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SOUTHERN UTILIZATION RESEARCH BRANCH

SOUTHERN REGIONAL RESEARCH LABORATORY

OILSEED SECTION

SPECIAL PROGRESS REPORT ON DEVELOPMENT OF EMULSIFIABLE FATS AND OILS AND FAT EMULSIONS FOR USE IN INTRAVENOUS ALIMENTATION FOR THE DEPARTMENT OF THE ARMY OFFICE OF THE SURGEON GENERAL

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PREFACE

At the request of Col. T. E. Huber of the Office of Surgeon General, dated January 9, 1956, this Special Report has been compiled for the purpose of providing:

1. An approximately chronological sequence of research emphasis on the fat emulsion program as it developed at the Southern Regional Research Laboratory;

2. The accomplishments which have strengthened or redirected the research program;

3. The current status of research;

4. The projected research necessary to develop a practical fat emulsion.

The Report consists of eight sections with appropriate sub-sections where needed, as is indicated in the Table of Contents. Section I is a brief summary of the results accomplished under this research program. Section II is an introduction which outlines the development of the research program on Fat Emulsions and indicates the importance of various aspects of the problem. Sections III through VI report the progress which has been made in developing emulsifiable fats and oils and fat emulsions for intravenous alimentation. A critical review of their status and prospects is presented in Section VII. Publications and Reports of this laboratory are listed in Section VIII. For ease of reading, each sub-section begins with a summary statement of the general nature of the research reported therein and the significance of the results obtained. Then follows a detailed account of experimental conditions and results. Finally, a concluding statement is provided to summarize the results obtained and possibly indicate the direction of needed additional effort. Literature references are listed at the end of those sub-sections in which they have been indicated.

This arrangement of the report permits the reader to select the amount of detail he wishes to read in accordance with his interest.

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I. SUMMARY

Although considerable research had been conducted for several years at a number of institutions prior to 1952, no practical emulsion for intravenous alimentation had been developed. Investigators did not agree upon the best source or type of oil to be used and oil was considered to be a critical factor. There was no systematic information available on emulsifiers, and very little on the relation between chemical and physical properties of emulsions and their *in vivo* performance. Since November 1952 considerable progress has been made in solving these problems and this Laboratory has contributed along the following lines:

Oils. We have prepared 10 different synthetic glycerides and 19 natural oils processed in various ways, all of known history or known physical and chemical characteristics. These materials were distributed to 10 research groups who have received a total of ca. 800 lbs. of laboratory processed and 1325 lbs. of commercially processed oil. The samples included pure 2-palmitodiolein, a butyro-olein product, aceto-olein, simulated human fats, lactyl glycerides, and cottonseed, peanut, olive, sesame, and rice oils. As a result of testing these materials the Task Group has now agreed to conduct work with a selected commercially available processed cottonseed oil. This oil appears to contribute a minimum of adverse responses on injection of the emulsion and use of this oil has reduced the number of variables under investigation - a definite contribution towards the solution of this problem.

Phosphatide Emulsifiers. All of the emulsions on which research was being conducted by the OSG Task Group contained a purified soybean phosphatide until we directed attention to the fact that phosphatides should be suspected as a possible source of adverse reactions. We developed and have applied chromatographic techniques to demonstrate that chemical changes involving phosphatides do actually occur during preparation and storage of emulsions. Research is being conducted to further characterize the products formed from the phosphatide component of emulsion and to establish whether they can be responsible for undesirable physiological reactions.

Lecithin. Since lecithin is the principal component of phosphatides, part of our work has been directed toward the preparation of a pure lecithin from egg yolk. We have prepared a lecithin fraction, free from cephalin, but still containing minute quantities of other materials. It is planned to further purify this fraction and test it as a component of fat emulsions.

Nonphosphatide Emulsions. As one approach toward the development of practical intravenous fat emulsions an intensive testing of various nonphosphatide emulsifiers which might be used was undertaken. Forty-six different emulsifiers have been screened for emulsifying properties in a total of 96 systems containing one or more emulsifiers. It was found that the fatty acid esters of glycol and glycerol, and the fatty amides imparted the greatest physical stability. The most promising of these emulsifiers have been incorporated into a total of 401 emulsions, of which fourteen of the most promising have been submitted to the Army Medical and Nutrition Laboratory for rat toxicity tests. Five were nontoxic to rats when injected at the 40 ml./kg. level. The systems which have passed those tests consist essentially of two or three emulsifiers (particularly TEM, and acetylated tartaric acid ester of monoglycerides, and DRUMULSE), produced by two commercial firms. These firms are cooperating with us in attempts to improve and better adapt their emulsifiers for this use. Preliminary tests indicate that a nonphosphatide emulsion is feasible and further development and animal testing are indicated.

Radiation Sterilization. Samples of fat emulsion were prepared, sent to the Quartermaster Corps, and subjected to radiation of 3 million reps. These emulsions were not adversely affected with regard to particle size or emulsion stability. They were not toxic when injected into rats at the 40 ml./kg. level. Further research is indicated and should permit the use of emulsifier systems not otherwise available because of instability to autoclaving.

Physical Characteristics - Particle Size. The particle size distribution in an emulsion is believed to be an important factor in clinical performance. Electrophoreograms of portions of a typical emulsion after centrifugation indicate that the particles are in at least two ranges of size. A majority of the particles are 0.5 - 0.7 micron in diameter and have a mobility about three times that of the few larger particles which are about 1.5 - 2.0 microns in diameter. Examination of a phosphatide-containing emulsion after passage through plastic recipient sets showed a definite increase in particle size in the first portion of emulsion to pass through.

Electrophoreograms of Serum and Serum-Emulsion Mixtures. The *in vitro* stability of various emulsions when mixed with serum is being determined by electrophoresis according to a procedure which gave the best protein fractionation. Change in the electrophoreograms of both human and rat serum proteins in the presence of various emulsions were noted. The most significant observation was that one fraction, α_1, was notably absent in all cases where the added emulsion contained soybean phosphatides as

the emulsifier, and in these mixtures the mobility of the fat was greater than that of the naturally occurring fat in normal serum. Future work will include quantitative determinations of the changes in each fraction of human and rat sera in the presence of various emulsions and study of the changes in dog serum patterns after the infusion of an emulsion.

Status and Prospects. We now have a program on fat emulsions, and more systematic information is available but the effort should be intensified. Research under way should give evidence as to utility of a pure lecithin in intravenous fat emulsions, and the effect of processing and aging on the chemical composition of phosphatide emulsions.

We have been able to make nonphosphatide emulsions that appear promising on preliminary animal tests. These should be further developed and tested with rabbits, dogs, and cats to establish that the fat is metabolized and that no harmful physiological effects are produced.

There is need for more fundamental physiological experiments in conjunction with the program of research on emulsion preparation and properties to determine what causes the undesirable physiological reactions that are sometimes observed. A joint effort by chemists, physiologists, and medical research men is required for ultimate solution of this problem. Work on the electrophoresis of serum and serum emulsion mixtures has indicated certain fundamental differences in different types of emulsions and their relation to serum proteins. The development of basic information using this technique should lead to preparation of emulsions which will behave with regard to serum proteins in the blood stream in a manner similar to the natural fat transported by the blood. We have had successful cooperation from members of the Task Group and anticipate even better cooperation in the future.

Although there is still no practical intravenous fat emulsion available, considerable progress has been made as indicated in this report. We are confident that further basic information will lead to the development of such an emulsion.

II. INTRODUCTION

The Oilseed Section of the Southern Regional Research Laboratory has received support from the Office of Surgeon General for approximately three years, for the development of emulsifiable fats and oils and fat emulsions for intravenous alimentation. Prior to this time, occasional requests to supply special oil samples had been received from several institutions who were under contract to the Office of Surgeon General. It was not until late in 1952, however, that the Office of the Surgeon General requested the Southern Regional Laboratory to become actively engaged in the fat emulsion program. The initial problem of this laboratory was the development of a suitable oil, preferably from a domestic source, for use in intravenous fat emulsions. Later the problem was extended to include the development of fat emulsions.

Importance of Problem. Intravenous fat emulsions when satisfactorily developed would provide a means for maintaining adequate caloric intake in those patients unable to take, retain or utilize sufficient nutrients by oral means. Presently-available intravenous fluids do not provide adequate calories for maintenance of a positive nitrogen balance and rebuilding of injured tissues. Military and civilian uses for such an emulsion would be great and it has been estimated that perhaps 10% of hospital patients might be given such an emulsion were it available.

It is now a routine practice to give protein transfusions to patients who have suffered loss of protein either through disease, surgery, battle casualty, or other causes. The theory of these intravenous feedings is that it is necessary to maintain a positive nitrogen balance in patients and a high level of protein availability for maintenance of blood volume and biological activity, rebuilding injured tissues, and maintenance of resistance to disease. This holds true whether the protein is given in the form of whole blood or blood plasma, blood plasma fractions, or hydrolysates.

There is a major difficulty with this treatment. Unless adequate calories are made available to the patient along with the added protein, most of the beneficial effect of addition of protein will be lost because the

protein will be used as a source of energy instead of as a source of amino acids to regenerate needed protein tissue. It is difficult to accomplish this by simultaneous feeding of sugar solutions because there aren't enough calories available by such a method to be given conveniently. Excessively large volumes or concentrations of sugar solutions would be required to supply the calories needed. However, the amount of calories available in suitable fat emulsions is such that small amounts of fat emulsions could be used along with protein feeding to supply the needed calories and therefore make the proteins available for what they were originally intended.

Many years of research have been applied to the preparation of a stable fat emulsion suitable for intravenous alimentation in humans. Among the more recent investigators, the Department of Nutrition of Harvard University has conducted research in the fat emulsion field and has many publications concerning their findings (see listing of Publications of Department of Nutrition Harvard School of Public Health 1942-1952). These and various other groups or individuals had been able to demonstrate the feasibility of preparation, administration and utilization of emulsified fats of plant or animal origin. B. G. P. Shafiroff and coworkers at New York University have numerous publications during the past ten years concerning infusion into humans of emulsions prepared from a variety of oils and emulsifiers. H. C. Meng and coworkers of Vanderbilt University School of Medicine have published over the past decade concerning emulsion preparation, administration, fat clearance, and utilization of the ingested fat in animals and humans. Meng's studies also include use of fatty acids. M. I. Grossman and coworkers of the Army Medical Nutritional Laboratory have done considerable work, reported in publications and reports, on administration and utilization of intravenous fat emulsions in man and to a lesser extent in animals.

At a conference held in the spring of 1951 under the auspices of the Surgeon General's Office and the National Research Council, Dr. K. S. Markley, then with the Southern Regional Laboratory, was present by invitation to comment on the use of vegetable oils for fat emulsions. As a result of his presence at this conference, Dr. Markley was invited to visit the School of Public Health at Harvard University to discuss further cooperation with this institution. On this visit, plans were made to supply the School of Public Health with several samples of acetoglycerides which would be tested in fat emulsions.

During the same period, arrangements were made with Professor H. C. Meng of Vanderbilt University to supply him with fully synthetic fats which presumably would not suffer from the difficulties encountered in using ordinary fats and oils in fat emulsions.

In March of 1952 it became necessary to investigate the status of research on fat emulsions in order to ascertain the results of investigations on the previously mentioned samples supplied by the Southern Regional Laboratory and to review the entire picture to see how the Laboratory could most efficiently be of help in solving this very important problem. A

thorough examination of the literature revealed the following information to be known:

a) It was possible to administer clincially some fat emulsions in some cases without visible ill-effects;

b) Animal experiments had been and could be used at least partially to indicate or counterindicate clinical use of various fat emulsion preparations;

c) Much additional information was needed on the various vegetable or animal fats and oils, natural and synthetic, which might be used in emulsions; on emulsifiers, either natural, modified or synthetic for use in preparing a fat emulsion; on utilization and tolerance of intravenous fat emulsions in animals and humans; and on methods for screening emulsifiers, fats, and oils for selection of the best possible materials.

To further evaluate the fat emulsion program, personal contacts were made by members of the staff of the Southern Regional Laboratory with those institutions receiving support from the Surgeon General. It was readily apparent that one possible point of confusion might exist because of the multiplicity of oils being used in emulsions, without complete knowledge of their history or purity. Olive, coconut, cottonseed, peanut, and sesame oils were being used or else had been used at some time. The conclusion reached was that the most valuable contribution of the Southern Regional Laboratory would be research on the relationship between the processing and purification of fats to their usefulness in intravenous emulsions. It was to conduct such a program that this Laboratory received its original support from the Office of Surgeon General.

III. OIL COMPONENT

The oil component of a fat emulsion furnishes the major portion of the caloric content of such a product and therefore is of fundamental importance. Its selection should be based on known history and purity, among other factors. One of the original problems of the fat emulsion program was the difficulty of obtaining a fat or oil of sufficiently high purity for intravenous injection as an emulsion. Oils obtained commercially, which have been processed by conventional mill methods in conventional mill equipment, contain naturally-present substances, such as unsaponifiable matter, pigments, etc., in addition to the glycerides, or possibly are contaminated by the processing methods employed. In either case, emulsion instability, pyrogenicity, and other undesirable reactions were experienced in the clinical evaluation of emulsions containing such oils.

Commercial processing methods eliminate or greatly reduce the content of non-glycerides in oils. Although such products are perfectly satisfactory for edible purposes, the presence of even trace amounts of non-glyceridic material in oils used for I.V. emulsions was suspected of causing the undesirable reactions. For this reason, a program of careful laboratory processing of selected oils by conventional as well as specialized procedures was undertaken.

Samples of 19 natural glycerides, representing 7 different oilseeds, and 10 synthetic glycerides were prepared, processed or synthesized and made available. A total of approximately 800 pounds of laboratory processed oils and 1325 pounds of commercial products were distributed to 10 different groups. Although not all the available oils were tested and complete reports were not obtained in all cases the data obtained indicated that the synthetic fats, e.g. synthetic "simulated human fat" and triolein, although utilized metabolically when administered in emulsions showed no significant differences from the better natural oils in the incidence of adverse clinical effects. A commercially available selected high quality cottonseed oil ("Wesson" oil) was found to be equal or superior to any synthetic or natural oil tested. This oil was adopted as the oil component for all emulsions in the OSG program and therefore eliminated one important variable.

The surface tension and interfacial tension against water of many crude and refined oils and synthetic glycerides were determined. The results showed that the interfacial tensions of the various refined oils and synthetic long chain triglycerides do not differ greatly and none are low enough to emulsify spontaneously without added emulsifier.

An account of these natural and synthetic oils, their physical properties, and the results of their physiological testing is presented in the following sections.

A. Natural Glycerides

A variety of natural oils were obtained and processed in the laboratory by various procedures. These oils were characterized and submitted for testing in fat emulsions. The oils included: a cold-pressed peanut oil extracted in our pilot plant from hand-picked peanuts of excellent quality; a prepressed peanut oil obtained commercially, a prepressed and solvent extracted peanut oil, and various cottonseed, sesame, rice, corn, coconut, and olive oils.

In processing oilseeds for oil recovery they are subjected to grinding, heating or cooking, and pressing or solvent extraction to remove the crude oil. Crude oils are settled or filtered to remove suspended meal particles but still contain free fatty acids, gums, and other impurities. Except for olive oil which is consumed as a crude oil, these oils are generally processed for edible use in the following steps:

1. Refining with alkali and centrifuging to remove free fatty acids (as soaps), gums or phosphatides and some color bodies.

2. Water washing to remove soaps.

3. Bleaching by heating with adsorbents to remove red, yellow and green pigments, i.e. carotenoids and chlorophyll-like pigments, and residual soap.

4. Deodorization by heating under high vacuum and stripping with steam to remove volatile and odorous impurities.

The processing of the different oils included alkali refining, bleaching, and deodorization, the processes being conducted in stainless steel or glass laboratory equipment. Some oils were winterized. This process involves removal of high-melting fractions which form as the oil is cooled. During all stages of processing the oils were protected from oxidation by an atmosphere of inert gas.

As each oil was ready for distribution, it was identified by a number preceeded by the letters "OSG". A descriptive sheet was prepared giving the type of oil, its characteristics, and other pertinent information. Various investigators were notified as to the availability of samples, and shipments arranged upon request. It soon became evident that more oil samples were being prepared than could be tested by the different investigators. Obviously, physiological testing requires more time than does the preparation of an oil. Another complicating factor was the tendency of different investigators to prefer different oils for thorough testing.

From tests reported, it can be concluded that peanut oil is not a good oil for use in intravenous alimentation. Even though this oil was prepared from the best grade of peanuts obtainable, was water-white in color and to an exceptional degree free of foreign matter, the oil appeared to produce adverse effects when emulsified and injected into animals. One group of investigators did claim that it was the best peanut oil they had tested, but it was not as good as other oils.

In the course of testing the different oils results were obtained, which were somewhat erratic but which indicated that most oils made suitable emulsions. Winterized cottonseed oil was one of the best oils according to these tests.

Additional tests with winterized cottonseed oil indicated a commercial product of this type, Wesson Oil, selected batches of which were supplied by the manufacturer, were as good as products prepared in the laboratory. Consequently, arrangements were made with the manufacturer to supply our needs of this oil. Use of this type of oil by all investigators in the OSG Fat Emulsion Task Group has eliminated oil as a variable and thus permitted concentration on other factors such as emulsifiers and emulsion properties which now probably are more significant than oil with respect to adverse clinical reactions. Further work on the modification of oils is being held in abeyance.

Description of the history and preparation of the natural oils which were processed and distributed follows.

<u>Cold-Pressed Peanut Oil (OSG No. 1).</u> The first oil to be processed and made available for testing in this program was a peanut oil from fresh prime peanuts. Particular attention was given to the best techniques and precautions as applied to the pressing of the peanuts and the subsequent purification of the oil by the methods of refining and bleaching.

The peanuts used were current crop U. S. No. 1 grade shelled Dixie Runners, obtained from Blueplate Foods. Immediately upon receipt, the peanuts were placed in drums and stored at 3° C.

Approximately 650 pounds of the peanuts were passed over a picking table and approximately 14 pounds of assorted twigs, broken and damaged kernels, etc., removed. The cleaned peanuts were replaced in drums and stored at 3°C. until processed.

The pilot plant equipment used to cold-press the peanuts included a French hydraulic press, cake former, and cracking and flaking rolls. This equipment was very thoroughly cleaned prior to use. New Nylon press cloths, which had been thoroughly washed in hot water, were used.

The peanuts were removed from the cooler and allowed to come to room temperature. They were then cracked and flaked to a thickness of 0.010-0.012 inches. The oil content of the flakes was 48.05%. The uncooked flakes were formed into cakes weighing about 8 pounds each, placed in the 6-cake hydraulic press, and pressed at 4400 psi gauge. Oil from the first pressing was used as a wash for the press and not added to the subsequent pressings. A drainage time of only 15 minutes was allowed, as percentage yield was secondary to possible oxidation of the oil. Oil from all pressings was collected in a large container for complete mixing. The oil was protected by a stream of nitrogen during the approximate 6 1/2 hours required for the pressing. The entire quantity of oil was filtered through a bed of diatomaceous earth (Filter Cel), and stored at 3°C. Yield of filtered oil, 23 gallons; average amount of oil retained in the press cake, 20.44%.

A 5-gallon quantity of the fresh peanut oil was refined in a stainless steel, steam-jacketed kettle. Throughout the refining, a nitrogen blanket was used to protect against oxidation. The refining data are summarized as follows:

Free fatty acids in the crude oil (as oleic), 0.2%

Weight of oil refined, 36 pounds 13 ounces

Excess sodium hydroxide, 0.4%

Strength of lye, 16° Bé

Total lye, 3.87% of 16° Bé

Stirring time at 26°C., 30 min.

Max. temperature, 64° - 65°C.

Time at max. temperature, 12 min.

Settling time hot, 60 min.

Settling time at room temperature, 60 min.

The refined oil was filtered through a bed of diatomaceous earth (Filter Cel), and stored under nitrogen.

The bleaching of this refined oil was also carried out in a nitrogen atmosphere. The details are summarized as follows:

Weight of oil, 33 pounds 6 ounces

Amount of bleaching clay (Bennett-Clark), 5%

Amount of activated carbon (Nuchar), 1%

Bleaching temperature, 90° C.

Time to reach temperature, 9 min.

Time at bleaching temperature, 5 min.

The bleached oil was filtered through diatomaceous earth (Filter Cel), but was not completely clear after the first filtration. A second filtration yielded a sparkling clear oil. The filtrations were not conducted under nitrogen, but the refined and bleached oil was stored under nitrogen. The free fatty acid content of the oil was 0.004%.

The processing details as given for this peanut oil were applied to all subsequent oils processed in the laboratory.

The peanut oil obtained had been carefully protected against contamination in all stages from prime whole peanuts to refined and bleached oil. It would not be practical to produce an oil under cleaner conditions.

Cottonseed Oil (OSG No. 2). Because it was thought at one time that a commercially refined and bleached cottonseed oil would not have the purity needed for intravenous use, a sample of crude cottonseed oil was refined in the laboratory by methods used commercially but under conditions which would reduce to a minimum any oxidation of the oil or contamination with foreign matter.

The crude cottonseed oil was obtained in a 55 gallon drum from the Swift & Co. mill at Harlingen, Texas. This was an expeller pressed oil and contained 1.8% free fatty acids (as oleic). The drum of oil was stored at about 56°F.

Five gallons of the oil were withdrawn from the drum, the contents of which had been thoroughly stirred. This oil was refined in a stainless steel, steam-jacketed kettle, under a protective blanket of nitrogen. Details of the refining are as given for oil OSG No. 1.

The refined oil was filtered through a bed of diatomaceous earth (Filter Cel) and stored under nitrogen.

The bleaching of the refined oil was also carried out in a nitrogen atmosphere. The bleached oil was filtered through a bed of diatomaceous earth (Filter Cel), and stored under nitrogen at 3°C.

The free fatty acids of the refined and bleached oil were 0.01%. The color was 35 yellow and 4.2 red, Lovibond scale.

Prepress Peanut Oil (OSG No. 4). This oil represents the commercial counterpart of the laboratory-pressed oil, OSG No. 1.

Commercial crude peanut oil, when available, has usually been through one or two settling stages, filtration, and storage in tanks, possibly in admixture with varying amounts of previously processed oils. It was deemed advisable to obtain a freshly pressed commercial peanut oil directly from the press, and thus of known origin, and process it in the laboratory, thus by-passing the above mentioned sources of possible contamination.

Crude unfiltered peanut oil was obtained in a 55-gallon drum at the Dothan Oil Mill Co., Dothan, Ala. This was an expeller oil pressed from culls and shrivels. The drum of oil was shipped to the Southern Regional Laboratory and allowed to settle for 1 week. The clear, crude oil withdrawn from the original drum contained 2.5% of free fatty acids (as oleic).

Five gallons of the crude oil was refined, bleached and deodorized as previously mentioned.

Solvent Extracted Peanut Oil (OSG No. 5). Also obtained at the Dothan Oil Mill Co. was a 55-gallon drum of crude peanut oil, solvent-extracted from the press cake mentioned above. This oil had a free fatty acid content of 3.4% (as oleic), when siphoned out of the drum at the Laboratory. The refining, bleaching, and deodorization of this oil was by the identical procedure as employed with the pressed oil.

Peanut and Cottonseed Oils Winterized in Crude State (OSG Nos.8,9,10). The winterization procedure accomplishes the removal of a portion of the saturated, high-melting glycerides from vegetable oils, whereby the solid point of the oil is lowered. Commercial winterization is preceded by refining and bleaching of the oil.

Refined and bleached peanut oil is rarely if ever winterized, due to the formation of gelatinous, difficultly filterable crystals on cooling. Consequently, peanut oil has a relatively high solid point. It is believed desirable to lower the solid point of peanut oil in order to improve its properties as a possible oil for intravenous alimentation, since oils for this use should be capable of being stored at low temperatures, without the occurrence of solidification. Since the winterization of refined peanut oil presents a serious filtration difficulty, the winterization of crude peanut oil was attempted, with considerable success.

Two crude peanut oils were used; namely, a cold-pressed oil from prime peanuts (used for OSG No.1) and a commercial crude prepress oil (used for OSG No. 4). In addition, the refined and bleached oil from prime peanuts was winterized in the same manner, as a control. The oils were winterized

in acetone-hexane solvent (85-15 wt. mixture) at an oil to solvent ratio of 40:60 (wt. mixture). The solution of oil in solvent was cooled overnight to about -18°C, the temperature of the mixture then raised to -14°, and the product filtered through a cooled funnel. The filtrate was diluted with the same solvent to an oil-solvent ratio of 1:8 (wt. mixture), cooled to -65°C, and filtered through a cooled funnel. The precipitated glycerides were washed 3 times with cold solvent, and then stripped of solvent.

The refined peanut oil used as the control required approximately 5 times the filtration time of the crude oils at the -14° filtration. The -65° filtration was at about the same rate for both the refined and crude oils.

Cottonseed oil presents no filtration difficulty during winterization. A sample of cottonseed oil was, however, winterized and fractionated in the crude state, in the same manner as were the crude peanut oils. Filtration rate was not improved.

Each of the winterized and fractionated peanut oils, and the winterized as well as the fractionated cottonseed oil was refined, washed, bleached, and deodorized in the usual manner. The finished products were given the following identification: from prime peanuts, OSG No. 8; from commercial prepress peanut oil, OSG No. 9; from commercial crude cottonseed oil, OSG No. 10.

The contents of free fatty acid and unsaponifiable matter in the prime peanut oil, prepress peanut oil, and commercial cottonseed oil were considerably lowered by the combined winterization - fractionation procedure. Further, the colors of the fractionated oils were much lighter than refined oils which were not fractionated.

Rice Oil (OSG No. 12); Sesame Oil (OSG No. 17); and Olive Oil (OSG No.19). Rice, sesame, and to some extent olive oils may be classified as minor vegetable oils, since they do not have the widespread usage of such oils as peanut and cottonseed. From the standpoint of intravenous alimentation, rice and sesame oils have had only limited usage, with the quality of the oils more or less unknown. Olive oil has enjoyed extensive usage, but in the crude state as a "U.S.P." product. As far as is known, refined olive oil, and rice and sesame oils of known processing history have not been employed in emulsions. For these reasons, it was desirable to process these oils in a careful manner, and have them evaluated physiologically as non-thermogenic products for use in emulsions.

The rice oil was obtained from the American Rice Growers Cooperative, Houston, Texas. It represented a "refined, partially de-waxed" oil, free fatty acid content, 0.85%. The virgin olive oil, "Freemont Trail" brand, was from a producer in California. The initial free fatty acid content was 1.4%. The sesame oil, free fatty acid content 0.9%, was a commercial crude oil of the solvent-extracted type.

The three oils were refined in similar manner, using 16°Be'. lye for the rice and sesame oils, and 18°Be'. lye for the olive oil. Stirring time in the cold was 30 min. for the rice oil, and 50 min. for the other two oils. At the completion of refining, each oil was filtered through a bed of diatomaceous earth (Filter-Cel).

Each of the three oils was bleached with 5% of bleaching earth and 1% of carbon. The bleached products were carefully filtered and stored under nitrogen until ready for deodorization. Deodorization was accomplished in the usual manner at 232°C. for 45 minutes. The samples were placed under nitrogen in sterilized bottles. Analysis for free fatty acid content of the finished oils gave a range of 0.05-0.06%.

Rice Oil Winterized in Crude State (OSG No. 13). A sample of rice oil was winterized and fractionated in the crude state, and then bleached and deodorized. No alkali refining was employed.

Crude Olive Oil (OSG No. 20). This oil was known to be an authentic, unadulterated virgin olive oil. It received no processing other than filtration.

Crude Rice Oil (OSG No. 21). The source and history of this oil was known. No processing was applied.

Processed Olive Oil (OSG No. 19). This sample represented an olive oil which had been refined, bleached, and deodorized in the manner described for the other oils. Olive oil generally is used for edible and medicinal purposes with little or no prior processing.

Winterized Olive Oil (OSG No. 22). A portion of the refined, bleached and deodorized olive oil identified as OSG No. 19 was, in addition, winterized under laboratory conditions.

Cottonseed Oil Stored as Crude (OSG No. 25). A program was outlined for determining the effect of storage of crude cottonseed oil on its properties after subsequent processing and emulsification. A very fresh, prime crude, prepress cottonseed oil was obtained from a known source, a portion processed immediately and distributed for physiological testing. Subsequently, two additional batches of processed oil were prepared from the original crude oil stored for periods of one and three months, respectively.

Cottonseed Oil, Fully Processed (OSG No. 26). A cottonseed oil for comparison with commercially processed "Wesson" brand oil was purified by fully processing (refining, bleaching, winterizing, and deodorizing) in laboratory equipment a fresh, crude, prepress commercial oil.

Non-Winterized, Commercial Cottonseed Oil (OSG No. 27). By special arrangement a batch of commercially refined, bleached and deodorized cottonseed oil was obtained and distributed as OSG No. 27. Since this oil was not winterized, it represented the commercial counterpart of the laboratory processed cottonseed oil identified as OSG No. 2.

Undeodorized Commercial Cottonseed Oil (OSG No. 28). A portion of cottonseed oil which was commercially refined, bleached, and winterized, but not deodorized, was obtained by special arrangement from a commercial source and deodorized in the laboratory. This oil was compared with a commercially purified and winterized cottonseed oil, "Wesson" Oil.

Selected, Commercially Processed Cottonseed Oil, Wesson Oil. As part of this program, it was important to determine whether a selected, commercial cottonseed oil, fully processed, could be obtained which in all respects would be equal to oil processed in the laboratory. Arrangements were made with a commercial supplier to obtain such an oil, "Wesson" Oil. This oil has been supplied in large quantity to many investigators.

B. SYNTHETIC GLYCERIDES

Ten synthetic oils of widely varying compositions were prepared. In all about 100 pounds of these synthetic glycerides, some with different combinations of the common saturated and unsaturated long chain fatty acids, some new compounds with known configurations, and other modifications, were prepared and supplied for the preparation of emulsions. These synthetic products which were successfully prepared were "simulated" human fat of various proportions of components, acetostearin, mixed acetoglycerides, lactyl monoglycerides, lactyl dipalmitin, palmitoyl dilactin, tripelargonin, aceto-olein, palmito-diolein, butyro-diolein, and dibutyro-olein. The preparation of palmitoylated dextran of polyhydroxystearic acid and some lactopalmitins was undertaken but was not completed because the difficulties encountered made their future use obviously impractical even if the preparations were successfully completed. The fact that the synthetic oils are metabolized when administered intravenously as emulsions was established and no significant differences were observed in the clinical effects produced by these oils and by comparable emulsions of natural oils, such as olive oil or cottonseed oil. Details of the preparation of the synthetic oils are given below.

"Simulated" Human Fat (OSG Nos. 3, 14, and 15). The first of these synthetic fats was prepared to "simulate" human fat in composition. Dr. Meng, University of Vanderbilt Medical School, had requested a linoleic acid-containing fat which would supply the normal requirements for this acid.

The average composition of the body fat of man consists principally of three saturated and three unsaturated acids, representing 97% of the total on weight percent basis. These are divided into approximately 35.5% saturated acids (3.5% myristic, 7% stearic, and 25% palmitic) and 61.5% unsaturated acids (6% palmitoleic, 46% oleic, and 9.5% linoleic.)

In order to simplify the preparation and improve stability a triglyceride having a composition of 60% oleic acid, 2.6% linoleic acid, 30.0% palmitic acid, and 7.4% stearic acid was prepared, using commercial Emersol 233 LL Elaine, Neofat 156, and Neofat 165 as sources of the fatty acids.

These commercial products were distilled in order to remove any impurities. The first fraction of the distillates amounting to approximately five percent of the total fatty acids was discarded. The pot residues amounting to about 20% of the total fatty acids were discarded also. Glycerol was esterified with the distilled fatty acids by esterification procedure described in a publication by Feuge, et al (1). Briefly, 3,953 grams of Emersol 233 LL Elaine, 1,666 grams of Neofat 156, 380 grams of Neofat 165, 704 grams of 95% glycerol, and 10.83 grams of $SnCl_2.2H_2O$ (catalyst) were reacted at a temperature of 200°C. and a pressure of 25 mm. Hg. for eight hours. The fat was refined as prescribed for Hydraulic or Hot Pressed Cottonseed Oil using 16°Be. lye. The fat and the lye were placed in a refining kettle and rapidly stirred for 15 min.

at 40°C. The temperature then was increased to 70°C. and the fat slowly stirred for another 15 min. After the soaps had settled the clear oil was decanted and bleached using 4% BC bleaching clay and 0.4% carbon for 30 min. at 100°C. with rapid stirring. The bleaching clay and carbon was removed by filtration and the fat was deodorized at 220°C. at 2 mm. Hg. for one hour using a laboratory steam deodorizer (2). Final yield of synthetic fat was 4600 grams.

Analysis of the fat gave the following results:

Peroxide value	1.0 meq.peroxide oxygen/kg.fat
Free Fatty Acid (as oleic)	.08%
Saponification value	200.5
Mean molecular weight	840
Unsaponifiable matter	.1%
Iodine value	53.7
Hydroxyl value	3.5
Specific gravity at 40°/4°	.8982
Refractive index at 40°C.	1.45848
Color (Lovibond)	2 Yellow/.47 Red

Five additional batches of synthetic "simulated" human fat of essentially the same fatty acid composition were made by this procedure, for a total of about 68 pounds.

"Simulated" Human Fat, Low Melting (OSG No. 23). To obtain "simulated" human fat of lower softening or melting point, a batch of the synthetic fat was prepared with an increased amount of unsaturated fatty acid. The quantities of fatty acids used were such that about 85% of the fatty acids of the fat were oleic acid, with a corresponding reduction in palmitic acid content. Three batches of synthetic fat of about this composition were prepared in a manner analagous to that described previously for OSG No. 3. Analytical data on these materials are as follows:

Analyses of Three Preparations of OSG No. 23

Batch	1	2	3
Amount, lbs.	6-3/4	10	11
Fatty Acid Distribution, %			
Oleic	79.66	79.66	84.75
Linoleic	1.97	1.97	1.44
Linolenic	0.78	0.78	0.79
Conj.dienoic	1.05	1.05	1.14
Conj.Trienoic	0.02	0.02	0.01
Sat'd.acids (Mostly Palmitic)	16.52	16.52	11.87
Color, Lovibond	10y/1.84r 1/	10y/1.58r 1/	5-10y/0.8r 1/
Free fatty acids %	0.06	0.04	0.02
Iodine value	75.2	75.8	79.4
Hydroxyl value	1.8	8.4	0.13
Refractive Index @ 40°C.	1.4615	1.4614	----
Sap. value	----	----	195.1

1/ y = yellow; r = red.

Tripelargonin (OSG No. 11). A simple triglyceride was prepared with pelargonic acid of about 99% purity. The fatty acid of this product had an odd number of carbon atoms and therefore differed from the other synthetic oils, all of which contained fatty acids of even numbers of carbon atoms. Ten pounds of Tripelargonin was prepared by esterification as described for OSG No. 3. The analysis of this material follows:

Color	Water White
Refractive Index at 25°C.	1.4480
Specific gravity at 24.7°C.	0.9423
M. P.	Approx. 8.5° C.
Free Fatty Acid	0.03%
Hydroxyl value	3.2
Saponification value	327.5

Acetostearin (OSG No. 6a). A mixed glyceride of a low and a high molecular weight fatty acid was prepared. This fat, acetostearin, was synthesized from commercial monostearin and acetic anhydride as follows: A molecularly distilled monostearin (Myverol 18-05) was crystallized twice from acetone at 0°C. The crystallized product contained approximately 97% monostearin.

One hydroxyl equivalent of monostearin (OH value 358) was reacted with two moles of acetic anhydride at 110°C. for one hour under an atmosphere of hydrogen. Acetic acid was removed by washing, and moisture removed. The product was bleached with 4% B.C. Clay at 70° for 10 minutes. Bleaching was followed by steam deodorization for 1 hour at 177°C.

The approximate composition of this product, calculated from the original composition of the monostearin and using the laws of probability, follows:

Glycerol monostearin	0.3%
Glycerol monopalmitin	0.1%
1,2-diaceto-3-stearin	50%
1,2-diaceto-3-palmitin	24%
1-aceto-3-stearin (diglyceride)	17%
1-aceto-3-palmitin (diglyceride)	8%

Aceto-Olein (OSG No. 6). Approximately 15 pounds of synthetic aceto-olein was prepared by reacting mono-olein and acetic anhydride. Mono-olein was prepared from distilled oleic acid which was converted into technical grade monoglycerides (minimum monoester content, 60%) by esterification with glycerol. The washed and dried monoglycerides were acetylated by reacting one hydroxyl equivalent with three moles of acetic anhydride for one hour at 110°C. Excess anhydride was hydrolyzed with water. After being washed and dried the acetoglycerides were bleached for 10 minutes with 4% neutral bleaching clay and 2% activated carbon at 70°C., and steam deodorized for one hour at 200°C. and one mm. mercury pressure.

Palmito-Diolein (OSG No. 7). Approximately 2.5 pounds of synthetic palmito-diolein of substantially known configuration was prepared from diolein and palmitoyl chloride. Intermediate preparations were methyl oleate, diolein, palmitic acid and palmitoyl chloride. Methyl oleate first was obtained from pecan oil by alcoholysis, and distilled under reduced pressure. The product was further purified by low temperature crystallization. Diolein was prepared by reacting 1 mole of the purified methyl oleate with 5/6 mole of glycerol in the presence of 0.1% sodium hydroxide at 250° C. The diolein was washed with acetic acid, then warm distilled water. The dried diolein was molecularly distilled.

Palmitic acid was prepared as follows: Methyl palmitate was prepared by esterifying Neofat 1 - 56 then distilling in a Podbielniak column. The fractionated methyl palmitate was further purified by low temperature crystallization, saponified, and palmitic acid recovered by acidifying the soaps. To prepare palmitoyl chloride, 0.8 mole of phosphorus trichloride per 1 mole palmitic acid was reacted. The palmitoyl chloride was stripped at room temperature, decanted, stripped at 100° C. for 1 hour, filtered and distilled.

Palmito-diolein was prepared by reacting 2.6 moles of the distilled diolein, 3.1 moles of the distilled palmitoyl chloride and 3.3 moles of pyridine in chloroform for 2 days under anhydrous conditions. The pyridine and chloroform were removed by washing with sulfuric acid and distilled water, then stripping. The product was washed with potassium hydroxide and water, bleached by adding carbon and B. C. clay, and filtered through filter cell.

The filtrate was deodorized at 180° C., 1 mm. pressure for one hour. The palmito-diolein was a clear, bright yellow liquid and analyzed as follows:

Percent Free Fatty Acid (as oleic)	0.24
Iodine Value	54.14
Melting Point	11.4 - 12.0° C.

Mixed Acetoglycerides (OSG No. 16). A 2 pound batch of mixed acetoglyceride was prepared by a procedure of interesterification of a good grade of refined vegetable oil with pure triacetin in the presence of sodium ethoxide.

Butyro-Olein (OSG No. 24). Approximately 12 pounds of a mixed butyro-olein was prepared by interesterification of methyl oleate and tributyrin. The methyl oleate was obtained as mentioned previously. The mixture of triglyceride was given a light refining, bleached, and deodorized at 200° C.

Mono-Olein (OSG No. 18). A small quantity, 15 gms., of pure 1-mono-olein was prepared by esterification of oleic acid with glycerol followed by molecular distillation and solvent fractional crystallization. This sample was requested for use in the preparation of radioactive glycerides.

Lactyl glyceride. The previously mentioned synthetic fats and oils were intended for use as the caloric component of I. V. emulsions without specific regard to their ease of emulsifiability. A lactyl glyceride was prepared, however, for the specific purpose of providing enhanced emulsifiability along with a calorie source.

Such a fat contains free hydroxyl groups and a low molecular weight fatty acid, both of which impart water affinity to the product, and hence ease of emulsification.

Several simple methods of preparation of dipalmitin (10, 11) and of lactyldipalmitin (including esterification (1), interesterification (16), and coupling halo-diglycerides (7, 8) with sodium lactate (9) and reactions of diglycerides with acid chlorides) were tried without success. The method which was finally successful and resulted in the preparation of beta-lactyl-alpha, gamma-dipalmitin was based upon a procedure reported by Feldmann and Fischer (2) and consisted of blocking the hydroxyl group of lactic acid with a benzyl group, converting the product to the acid chloride, coupling that with pure diglyceride (dipalmitin) and then hydrogenating off the benzyl group leaving the desired lactyldipalmitin.

The preparation and properties of beta-lactyl-alpha, gamma-dipalmitin were reported in a publication in the Journal of the American Chemical Society (3).

Because the melting point of lactyl dipalmitin was higher than room temperature (about 50° C.) and to compare the properties of synthetic glycerides having 1 and 2 lactyl groups, a small quantity of alpha-palmitoyl-beta, gamma-dilactin was prepared.

This was prepared in essentially the same way as was monolactyl dipalmitin except that the o-benzyllactyl chloride was reacted with monopalmitin. The alpha-palmitoyl-beta, gamma-dilactin had a lower melting point (33° C.) and a lower interfacial tension against water than does lactyl dipalmitin.

The pure lactyl dipalmitin and palmitoyl monolactin were tested as emulsifier-calorie materials and were found to be only fair to poor as emulsifiers in preliminary emulsion screening tests.

Lactyl monoglycerides. A series of products having potential emulsifier properties were prepared by esterification of a commercial monoglyceride with commercial lactic acid in various proportions.

The series of lactyl glycerides, i. e., lactyl monostearins, consisted of four compounds containing approximately 1, 3, 6, and 9 lactyl groups per monostearin molecule. This type of product might be considered analogous to a present commercial emulsifier, namely TEM, which has shown promise for use in this application.

The compounds varied from fair to good as emulsifiers in unautoclaved emulsions but were heat sensitive and the emulsions broke during autoclaving. The preparation of these materials is described below.

Monoglyceride, toluene (150-250 ml. per mole of lactic acid), and the 85% lactic acid, in molar ratios ranging from 2/1 to 16/1 of lactic acid to monostearin were used depending upon the lactyl content desired in the final product. The mixture was warmed and stirred until the monoglyceride dissolved and then the esterification catalyst was added (2 g. p-toluenesulfonic acid, or the equivalent of the Dowex-50 resin, per mole of lactic acid employed). With continued stirring the mixture was heated to reflux. When the desired amount of lactic acid appeared to have reacted, the reaction mixture was diluted with hydrocarbon, the resin catalyst filtered off and the acidity determined. With the esterifications employing sulfonic acid catalyst alkaline or $NaHCO_3$ washes followed by water washes were made, but in the others only distilled water washings were done. The solutions were dried over Na_2SO_4 and stripped under vacuum with nitrogen to the final products.

Analytical data indicated the four lactyl-monostearins to contain approximately 1, 3, 6 and 9 lactyl groups per monostearin molecule.

The four lactyl-monostearins were screened as emulsifiers. Two emulsion tests were made with each material namely, 1)-a simple stirring-screening test to observe and compare separation rates, and 2)-a test emulsion employing a "standard recipe" made in the usual manner, subjected to the usual treatments, and tested to determine the emulsions' qualities.

The results of the simple stirring-screening tests indicated the tri-lactyl-monostearin material appeared better than the best reference emulsion (Brij + F-68 combination at that time), the hexa-lactyl material not as good as this reference but better than the then second best reference emulsion (TEM + F-68), while the mono- and nona-lactyl materials were not equal to this second reference emulsion.

A 15% olive oil emulsion (without dextrose) made with 0.5% concentrations of both the mono-lactyl material and Pluronic F-68 was prepared and autoclaved, but did not pass the shaking and serum clumping tests. Later, one sample 15% cottonseed oil emulsion was made with each of these four lactyl-monostearins using 15% oil, 1.2% emulsifier material, 0.3% Pluronic F-68, and 5% dextrose solution. However, these test emulsions, after homogenization, separated into two layers during the autoclaving step of the emulsion preparation procedure.

Polyhydroxy acids. In addition to the synthetic fats and oils which were prepared for possible use as the oil component of I. V. fat emulsions, there has been considerable interest in the possibility of using synthetic water soluble materials of high caloric value for intravenous preparations. One such material which was of interest was the glyceride of a polyhydroxylated fatty acid.

Swern (4) reported the preparation of a diepoxystearic acid by epoxidation of linoleic acid. Mack and Bickford (5) were able to hydrogenate monoepoxystearic acid, prepared by the epoxidation of oleic acid, to 10-hydroxystearic acid. The preparation of a trihydroxystearic acid by epoxidation of linolenic acid and catalytic hydrogenation of the resultant triepoxide was therefore attempted.

The diepoxide from linoleic acid was successfully prepared and hydrogenated in this laboratory, although the hydrogenation proceeded much more slowly than with the epoxide of oleic acid. However, difficulties were encountered in the epoxidation of both linolenic acid and methyl linolenate. A pure triepoxide was not isolated from any of the reaction products. Catalytic hydrogenation of these epoxidized samples proceeded extremely slowly. The preparation of a trihydroxystearic acid by this method was therefore not considered practicable.

A sample of another trihydroxystearic acid (9,10,12-trihydroxystearic acid) prepared from ricinoleic acid, was purified and supplied for testing.

Palmitated Dextran. Since dextran is a polyhydroxy material which can be introduced into the blood without ill effects, the addition of some lipophilic groups, i.e. fatty acid, to the molecule should impart some of the desired properties. To obtain the desired emulsifier properties with dextran the acylation of only one hydroxyl group of every 5 to 10 groups in the molecule was considered necessary. Therefore the object of these experiments was to partially acylate, specifically palmitate, dextran to the extent needed to impart lipophilic properties to dextran.

Experiments to directly esterify dextran in an inert medium using reactant quantities intended to partially palmitate dextran proved unsuccessful as did the interesterification of dextran with hydrogenated cottonseed oil. A second interesterification trial employing the procedure of Lundberg and Chipault (12) indicated that some reaction proceeded but to a very minor extent.

Reactions were carried out with dextran and palmitoyl chloride in formamide (13) in dimethyl formamide, and in an inert solvent (14) following procedures of acylation indicated in the references cited. These did not yield the desired partially palmitated dextran but yielded a tripalmitated dextran whose properties were not suitable for use as a component of intravenous fat emulsions.

Conclusions. Many synthetic fats and oils were prepared and some were clinically tested in emulsions. Available results have established the fact that the synthetic oils which were tested are utilized metabolically when administered in emulsions and that no significant differences were noted in the clinical effects produced by these or natural oil emulsions. Since synthetic oils have not been found superior to natural oils at this time, further preparations are being held in abeyance.

The synthetic materials prepared for use in enhancing or promoting emulsification proved, in most cases, too costly or time consuming and were generally not even of equal value for this purpose over the presently used emulsifiers. In addition, progress made on investigation of non-phosphatide emulsifiers, which are commercially available, is very promising so that intensive investigation of synthetic materials or compounds suitable for emulsifiers does not seem warranted at this time.

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C. PHYSICAL PROPERTIES

One of the properties which an oil for use in emulsions must have is ease of emulsification. With given amounts of emulsifying agents and water, it theoretically is possible to emulsify different oils to different degrees. Since the interfacial tension of a liquid is a measure of its free surface energy per one square centimeter of interface, determination of interfacial tension provides information as to ease of emulsification.

The surface activities of simulated human fat, and of cottonseed, sesame, rice, olive, coconut, pecan, and peanut oils, in the crude, refined, and variously-processed stages were investigated. The significant results observed were that the interfacial tensions of the crude oils were in every case lower than those of the refined oils. Crude rice, cottonseed, and olive oils were significantly lower than the other crude oils. Among the refined oils the difference in surface activity is not very great. Since the refined oils do not differ to a great extent among themselves in surface activity, it makes little difference which is used in preparing emulsions. Crude oils are considered to be unsatisfactory because of their possible content of uncharacterized components.

The surface activities of several synthetic glycerides also were determined, including the glycerides of acetic, butyric, caproic, palmitic, oleic, pelargonic, and stearic acids. The interfacial tensions of the aceto compounds were lowest; of the other materials, about equal to natural glycerides.

The apparatus and method of determining surface and interfacial tensions is that which was first proposed by Allan Ferguson, (1) and later modified (2). Essentially, the method consists of measuring the pressure required to force the meniscus of the product at the end of a capillary tube from a curved to a plane surface. Calculation of surface tension is then made by the equation

$$\text{surface tension} = 1/2(r\ d\ g\ h)$$

where r is the radius of the capillary, d the density of the manometer liquid, h the actual pressure applied and measured on the manometer, and g the acceleration of gravity.

The surface phenomena of the various natural and synthetic oils were determined, and the results published in 2 articles (3,4). Summaries of these results are given in Tables I and II. (Tables I and II follow on pages 25 and 26.)

Conclusions. Thermodynamic calculations indicate that an oil must have an interfacial tension against water less than 1 dyne per cm. to emulsify spontaneously. However, if the droplets of oil in water are to have diameters of 0.5 micron, as required in emulsions for intravenous injections, the oil should have an interfacial tension against water less than 0.1 dyne per cm. In all oil in water emulsions investigated, the oil droplets are

TABLE I

Surface and Interfacial Tensions of OSG Oils

Oil	Equation for surface tension a/ dyne/cm.	Interfacial tension at 25° dyne/cm.
Refined peanut, solvent extracted	-0.0559t + 31.94	18.50
Refined peanut, cold press	-0.0637t + 32.00	22.87
Refined peanut, pre-press	-0.0753t + 33.61	18.12
Refined cottonseed	-0.0464t + 31.98	14.91
" rice	-0.0474t + 31.69	16.25
" sesame	-0.0641t + 31.30	17.66
" olive	-0.0492t + 29.78	17.63
" coconut	-0.0599t + 28.49	12.86
Simulated human fat	28.82 b/	18.55
Crude peanut, cold press	-0.0617t + 29.06	19.93
Crude cottonseed	-0.0283t + 27.99	1.3 c/
" rice		-6.30, 9.30 c/
" olive	-0.0679t + 32.55	6.3
" pecan		14.15

a/ Where "t" is in °C.
b/ Surface tension determined at 55° C.
c/ Anomalous results apparently are due to solubility effects.

TABLE II

Surface Tension Equations, Interfacial Tensions, and Changes in Enthalpy of Synthetic Glycerides

Name of Compound	Equation for surface tension a/ in dyne per cm.	Interfacial tensions in dyne per cm. 25° C.	75° C.	Change in enthalpy, ΔH in ergs
Triacetin	$\gamma = -0.10150t + 37.18$	3.33	----	64.89
Tributyrin	$\gamma = -0.07955t + 29.57$	11.90	----	51.28
Tricaproin	$\gamma = -0.05797t + 27.48$	18.56	17.32	43.30
Tricaprylin	$\gamma = -0.06252t + 26.93$	14.60	14.16	44.00
Tripalmitin	$\gamma = -0.06403t + 28.97$	----	11.01	46.45
Tristearin	$\gamma = -0.06778t + 30.96$	----	10.43	49.46
Triolein	$\gamma = -0.06933t + 30.10$	14.61	13.47	49.03
Trielaiden	$\gamma = -0.06986t + 27.18$	----	11.56	46.25
Trilinolein	$\gamma = -0.02867t + 20.85$	13.16	----	28.68
1,2 Diaceto 3 olein	$\gamma = -0.05330t + 22.95$	7.46	----	37.50
1,2 Diaceto 3 stearin	$\gamma = -0.09338t + 32.00$	----	2.90	57.50
1 Monostearin	$\gamma = -0.05876t + 24.52$	----	----	40.46
1 Monopalmitin	$\gamma = -0.06678t + 27.46$	----	----	45.69
1,3 Dipalmitin	$\gamma = -0.04625t + 25.85$	----	----	38.48
1,3 Dioleopalmitin	$\gamma = -0.04442t + 27.38$	14.50	12.26	39.51
Tripelargonin	$\gamma = -0.05650t + 25.38$	20.16	----	40.81

a/ Where "t" is in °C.

negatively charged and there is usually about 0.05 volt difference in potential between the oil and water phases. In the naturally occurring chylomicron emulsions, it is believed that the droplets of oil are surrounded by a protective layer of serum albumin and serum globulin. This protective layer gives the chylomicron emulsion an isoelectric point between 4.6-5.4.

Even though the interfacial tensions of the triglycerides vary with chain length, degree of unsaturation and type of geometrical isomerism, none of the triglycerides investigated have interfacial tensions against water small emough to produce the desired emulsions without the aid of an emulsifying agent.

Either a strongly polar group, such as $\overset{\text{O}}{\text{C}}\text{—}\overset{\text{H}}{\text{N}}\text{-}$, substituted on the triclycerides, a protective agent, such as the sera albumin and globulin, or emulsifiers will be required to give the triglycerides low enough interfacial tensions to form the desired emulsions.

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D. PHYSIOLOGICAL TESTS

Physiological testing of the natural and synthetic oils has been rather slow because of the expense and difficulty involved in testing these products. Not all of the materials furnished as OSG samples have been tested. Some results which are available, however, indicate that an oil is available for satisfactory use in emulsions for the present. Investigators at Harvard University found that the OSG samples of cottonseed oil produced the least amount of reactions of those oils they have tested when injected as an emulsion.

Results obtained thus far have indicated that fats and oils for intravenous alimentation fall into three groups. The first comprises oils which for one reason or another are not pure and give bad reactions when injected. The second group comprises most of the fats tested. These fats can be given to most patients satisfactorily; 70 to 80% of the patients receiving these emulsions have no reactions in terms of rise in temperature or back pains. The synthetic fats prepared at this laboratory as well as many of the natural oils, such as olive, coconut, sesame, and cold-pressed peanut oil belong to this group. The third group comprises the cottonseed oils that gave the minimum percentage of reactions. Investigators at

Harvard University found that cottonseed oil when injected as an emulsion into patients gave very minor reactions, 95% of the patients not having any reactions at all with only 5% having back pains and slight temperature rises. This remarkable result focused interest and attention on cottonseed oil and led to the development of extensive programs in cooperation with Harvard University, Vanderbilt University Medical School, Upjohn Company, Baxter Laboratories, the Army Medical Nutrition Center at Denver, Colorado, and the Army Graduate School at Washington, D. C. This program, on a large commercial scale, involved the use of 90 gallons of commercial cottonseed oil of high purity. This oil was made into emulsions by the Upjohn Company and Harvard University and tested clinically. To insure uniform oil of high purity arrangements were made by this laboratory with the Southern Cotton Oil Company to use selected samples of their best grade salad oil in making up the large quantities of oil now being tested.

Conclusion. As a result of a limited number of tests, it was found that intravenous fat emulsions of carefully purified cottonseed oil when administered clinically gave the least number of reactions compared to other purified oils under the same conditions. Moreover, it was found that the use of a selected high quality commercial cottonseed oil "Wesson" brand, is equally satisfactory to the laboratory-prepared purified cottonseed oil. In order to minimize the complexity of the problem and to eliminate for the time being one of the variables, other investigators in the Intravenous Fat Emulsion Task Force were encouraged to confine their emulsion testing to the use of commercial cottonseed oil which was supplied by one commercial firm working very closely with members of the staff of the Southern Regional Research Laboratory. Whether or not cottonseed oil will in the last analysis remain the preferred oil for use in fat emulsions cannot at this moment be predicted. When more pressing problems, particularly those involving the purity and reactivity of the emulsifiers and physical characteristics of the emulsions, are eliminated or greatly simplified, it may be possible to turn again to the oil and carry out more stringent evaluations of the various natural and synthetic oils.

IV. EMULSIFIERS

The original objective of the research on fat emulsions at the Southern Regional Laboratory was limited to the development of suitable fats for intravenous use.

In the course of work toward this objective it was found that laboratory processed winterized cottonseed oil, or a selected commercial cottonseed oil ("Wesson" oil), when tested clinically in intravenous fat emulsions gave fewer adverse reactions than other oils tested. Agreement on use of this oil in further research eliminated oil as a variable and permitted redirection of effort. The emulsifier system had proved erratic in its effect and the improvement of emulsifier systems was undertaken. The resulting work on emulsifiers may be divided into three areas: (1) purifying, characterizing, and testing phosphatides, (2) screening commercially available non-phosphatide emulsifiers, and (3) investigation of the physical properties of the emulsifiers.

Soybean phosphatides which are currently used in emulsions are a mixture of a number of compounds. Reproducibility of such phosphatide mixtures is difficult to attain. Also, the individual compounds in the mixtures degrade easily. Little is known about the surface activity of the individual compounds. To clarify the situation with respect to phosphatides, certain of the components were isolated. Their ability to form emulsions and their susceptibility to deterioration are being investigated.

In the area of non-phosphatide emulsions, approximately 46 emulsifiers were obtained from commercial firms. These emulsifiers were tested individually and in various logical combinations. Because of failure to form suitably fine oil particles, instability of emulsions on heating or shaking, or other reasons, most emulsifier combinations were found to be unsatisfactory. However, as a result of the testing program some predictions can now be made as to the probable performance of emulsifiers not yet tested. Most important, a few emulsifier systems were discovered which warranted further testing.

A. PHOSPHATIDES

Phosphatides are widely distributed in nature, being found in both the animal and plant kingdoms. Of the naturally available phosphatides only those derived from soybeans, cottonseed, egg yolk, and yeast were used in our investigations.

The phosphatides from egg yolk were fractionated and purified to obtain a pure lecithin, whose characteristics were determined. It was found that lecithins in the pure state are very hygroscopic and apparently quite sensitive to air and heat. Emulsification tests indicated pure lecithins were less efficient as an emulsifying agent than were mixtures of phosphatides which are normally used.

Soybean Phosphatides. The largest amount of effort was devoted to phosphatides from soybeans. A number of the reported procedures for preparing or purifying natural phosphatides involve solution in one solvent, such as diethyl ether or petroleum ether, and precipitation by addition of another solvent such as acetone or alcohol (1,2). Other variations include the extraction (2,3) of certain compounds with solvents or partitioning of fractions between solvents. By such procedures free or weakly complexed sugars, etc., are removed to yield purer materials. In the literature are recorded discussions and data which conflict and as many reviews point out, there exist numerous instances where further work is needed to clarify a particular point in phosphatide chemistry or technology.

For our work crude soybean phosphatides were obtained which were fresh and had received a minimum of heat treatment. A simple separation or purification was employed in which the whole phosphatide mixture was dissolved in petroleum ether and the phosphatides precipitated by addition of acetone. By this method some of the non-glyceride impurities are kept in solution; precipitated material contains a high concentration of phosphatide and is nearly oil-free. For a higher degree of purification this procedure was repeated several times. Following this solvent purification, the phosphatides were used in storage studies, in investigation of browning reactions in emulsions, and in other investigations as will be discussed later.

Cottonseed Phosphatides. Cottonseed gums, now being produced commercially, are a potential source of cottonseed phosphatides. The absence of linolenic acid in cottonseed phosphatides presents a point of significant difference from soybean phosphatides and should contribute to greater stability. The purpose of this investigation was to show that phosphatides could easily be isolated from crude cottonseed gums after removal of the gossypol as dianilinogossypol. Fractionation of the phosphatide portion on an alumina column (4) yielded a lecithin fraction free of cephalin.

To prepare the cottonseed phosphatides, a 627 g. sample of cottonseed gums was shaken with 1250 ml. of diethyl ether, and the aqueous layer was discarded. After filtering the ether layer, an excess of aniline (67 ml.) was added and successive crops of dianilinogossypol filtered off at room temperature, and discarded.

The ethereal solution was treated with acetone to precipitate the phosphatides. The precipitated material was leached with 95% ethanol containing 0.1% hydroquinone as antioxidant. Further fractionation of the alcohol soluble phosphatides was accomplished by chromatography on an alumina column. A total of 12.8 g., or about 58% of the material put on the column, was eluted in eleven 250-ml. fractions. The eluate varied in color from brick red in the earlier fractions to colorless in the latter ones, and after removal of the solvent the product was a pasty solid similar in appearance to egg lecithin.

Amino nitrogen was present in the crude cottonseed phosphatides but was not detectable by the ninhydrin test in the fractions eluted from the column. The fractionated material also gave a negative test for free fatty acid and a positive test for unsaturation, choline, and phosphate at a single spot characteristic of lecithin on paper chromatograms. This cottonseed phosphatide has not as yet been tested as an emulsifier.

Pure Lecithin from Egg Yolk. A literature survey of phosphatides as applicable to fat emulsions for intravenous use confirmed a popular opinion expressed at one meeting of the Task Force, namely, that more information was needed concerning phosphatides. Since lecithin was considered the most significant component of the commercial mixture of phosphatides currently used, a study of lecithin's role and eventual fate in emulsions was begun. A pure lecithin was prepared and its behavior and decomposition under conditions existing in emulsions were observed.

Preliminary investigations of the purification of phosphatides indicated that more precise and detailed procedures had to be followed to obtain pure lecithin for these studies. Accordingly the method of purification of lecithin from egg yolk as described by Hanahan, Turner, and Jayko (4) and Hanahan (5) was chosen for the isolation of pure lecithin. The procedure used is described as follows:

The separated yolks from three dozen fresh eggs were extracted with two volumes of acetone (Baker's reagent grade) for about 3 minutes in a Waring blendor while under a blanket of carbon dioxide.

The mixture was filtered and the filtrate was saved. The residue, which was kept under carbon dioxide, was extracted with two volumes of purified, 95% ethanol by allowing the ethanol-residue mixture to stand for three hours at room temperature. The mixture was filtered, the filtrate saved and the residue extracted again with one volume of ethanol, after which the residue was discarded.

The ethanol and acetone filtrates were combined and stripped with nitrogen at 45°C. while under vacuum, and then extracted successively with 400 ml., 400 ml. and 200 ml. of petroleum ether while under carbon dioxide, filtered and reduced to a small volume (250 ml.) under vacuum at 45°C. The concentrate was added to 4 volumes of acetone with agitation and after good mixing allowed to settle. The clear supernatant liquid was decanted, the gummy precipitate transferred to a Waring blendor, washed with 500 ml. acetone, decanted and re-washed with six 250-ml. volumes of acetone in a like manner. The precipitate was dissolved in petroleum ether, filtered if necessary, the volume reduced to about 150 ml., and the acetone precipitation and washing procedures repeated as above in the Waring blendor. Solvent was stripped off under vacuum and dry nitrogen at 40°C.

The isolation of lecithin from this phosphatide mixture was accomplished by chromatographic techniques as described by Hanahan (4). Batches of the egg phosphatides were passed through a chromatographic column. The absorbent was Merck's chromatographic grade alumina. A slight positive pressure of carbon dioxide was maintained on the system at all times.

For a typical chromatographic separation a 22 g. batch of crude egg phosphatides was added to a fresh column as a 3% phosphatide solution in 95% ethanol and eluted with 95% ethanol. The material began appearing in the third (750-1025 ml.) fraction and apparently was substantially eluted when 3275 ml. had passed through the column, as the next 3 fractions (250 ml. each) gave the same weight of residue as the third fraction. The elution was stopped after 4550 ml. had been passed through the column. The material considered as good lecithin amounted to 11.1 grams and appeared in the fractions from 1025-3275 ml. The material from these fractions showed good choline spot tests and did not give a positive spot test for amino nitrogen by the "ninhydrin" spot test procedure, which had been calibrated to be positive in the presence of only a few tenths percent of cephalin.

For the final purification, egg lecithin recovered from the chromatographic columns was combined, stripped of ethanol under nitrogen and vacuum at 40-42°C. The recovered material, weighing 29.8 g., was dissolved in diethyl ether, divided equally into two 250 ml. centrifuge bottles, and the volume in each bottle adjusted to about 30 ml. Then with stirring 4 volumes of acetone was added to each, a very milky mixture being observed when about 25 ml. of acetone had been added after which the mixture gradually became lumpy. No precipitation occurred as the last 30-40 ml. were added. The bottles were then centrifuged at 5°C. and 1500 RPM for about 0.5 hour. The clear liquid was then decanted, the precipitate dissolved in diethyl ether, diluted to 30 ml., and precipitated as before with acetone. This procedure was repeated four times, then the ether solution was filtered, after which a final ether-acetone precipitation was made. The precipitate was placed in a vacuum desiccator over phosphorus pentoxide and held at 0-5°C. The final weight of pure egg lecithin was about 22 grams.

The crude egg phosphatide was a light yellow pasty or waxy material and after chromatographing was very light yellow or nearly white in color. The final purified egg lecithin was a white to slightly yellow or tan colored waxy paste and after drying appeared to become a light tan color and somewhat harder material.

In a subsequent purification 10 dozen eggs were used, from which was obtained 150 g. of the crude egg lecithin. About half of this material was purified chromatographically.

Analytical data on the pure egg lecithin are listed in Table I. (Table I. follows on page 33) The analyses of other "lecithin" materials are included for comparison. These materials are soybean phosphatides purified by The Upjohn Co., the type used in their emulsions, and Asolectin, a partially purified soybean phosphatide containing about 3% of oil.

The highly purified egg lecithin analyzed 15.00% choline (theory for pure stearoyllinoleyl lecithin is 15.10%), while the commercial "lecithins" analyzed about 4%. This latter figure corresponds to an actual lecithin

TABLE I

Summary of Analyses of Egg Lecithin and Other "Lecithins"

Analysis	Egg	Theory for pure lecithin*	Asolectin	Upjohn purified	chromatographed and reprecipitated
mental Carbon, %		65.72	61.34	60.35	63.31
rogen, %		10.78	9.62	9.40	10.58
sphorous, %		3.86	3.10	3.17	4.20
rogen, % (Total)		1.74	1.07	1.10	1.99
io Nitrogen:Phosphorous		1.00	0.77	0.77	1.05
no-Nitrogen nhydrin)		0	+	+	Neg.
line		15.10	3.92	4.07	15.00

inoleylstearoyl lecithin.

content of about 25-30% for the commercial "lecithins" or phosphatides. Both samples of soybean "lecithins" gave positive reactions for cephalin but the pure egg lecithin gave a negative reaction.

Subsequent chromatographic analyses indicated that the alumina purified egg lecithin contained several choline-containing impurities. Another purification procedure (6) using silicic acid as chromatographic medium was reported to separate the cephalins and lecithins from small quantities of lysophosphatides. Employing this procedure with egg lecithin, fractions were obtained which when chromatographically analyzed showed single positive tests corresponding to that of cephalins or lecithins respectively. By modifying the eluting procedure it was possible to concentrate each component in certain fractions.

The commercially available silicic acid used in this chromatographic separation had a considerable amount of fine particles (a minimum of 40% passes 400 mesh) giving undesirable flow rates even with greatly increased pressure on the column. Since sieving proved impractical, a method was devised to remove some of the finest particles (about 5 microns in size) by a fluidized-solids technique. Such a partially "de-fined" silicic acid, in which about 20-25 wt. % had been removed, gave greatly improved flow rates (approximately 10 times greater) and apparently equivalent resolution of the phosphatidic components of the crude egg lecithin as judged by other chromatographic tests.

One of the important properties of an emulsifier is its behavior in an actual emulsion preparation. An emulsion was prepared with some of the egg lecithin purified by the chromatographic procedure of Hanahan et al (4). The emulsion consisted of 15% cottonseed oil (Wesson Oil), 1.2% purified egg lecithin, and 0.3% Pluronic F-68 in 5% dextrose solution, and was prepared by high-pressure homogenization.

The emulsion preparation was not an acceptable one by the usual standards because it was of larger particle size (up to 7 microns) than a normal phosphatide emulsion prepared in the same manner. On autoclaving the emulsion broke slightly, with approximately 3-5% of the oil separating. The pH of the unautoclaved emulsion was 4.25, and after autoclaving was 3.85. The fatty acid content after autoclaving was about 0.1% (as oleic).

The purified lecithin before use in this emulsion had been kept in the dry state over P_2O_5, under vacuum, at 0^oC. for about ten weeks during the determination of analytical and other values. Visual observation indicated it had darkened considerably and acquired a strong odor during this period, and in view of difficulty experienced in preparing the emulsion it was decided that this first emulsion preparation was not a conclusive test of pure lecithin.

A second emulsion test was made with egg lecithin which was subjected to the final purification steps and dried under vacuum overnight immediately prior to its use. The 15% Wesson oil emulsion was made by dispersing the purified lecithin (1.2%) in Pluronic F-68 (0.3%) dissolved in 5% dextrose

solution. Although good particle size (mostly less than 1 micron but a very few up to about 8 microns) was obtained in only one pass through the homogenizer, the emulsion was sensitive to sterilization and even the unautoclaved emulsion began creaming on overnight standing. The pH (4.85) was essentially unchanged on autoclaving. Lower concentrations of lecithin and/or Pluronic F-68 might improve the stability of the egg lecithin emulsion.

Yeast Lecithin. A purified sample of yeast lecithin was obtained from Dr. D. J. Hanahan. This material was used for comparison of its chromatographic characteristics with those of the pure egg lecithin. The results obtained are discussed in Section VI under Chromatography.

Conclusions. Certain properties of phosphatides which might affect their use in fat emulsions for intravenous administration have been investigated. Soybean phosphatides are gross mixtures containing only about 25% of lecithin,and simple purification procedures such as solution followed by precipitation do not always accomplish the same degree of purification. Lecithin has been isolated from cottonseed gums and such a lecithin may be expected to be more stable than soybean lecithin by reason of the absence of linolenic acid. Pure lecithin was isolated from eggs and some of its properties and chromatographic characteristics were determined. Emulsification tests indicate that pure lecithin is less efficient as an emulsifier and is less stable than the soybean phosphatide mixtures now being used. Techniques have been developed which make possible better characterization of phosphatides which can now be applied to control of purity in phosphatide preparations. Research on preparation of pure and well-defined phosphatides can now proceed at a greater pace and should be continued.

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B. NON-PHOSPHATIDE EMULSIFIERS

There is no evidence at present to show that phosphatides are a necessary component of fat emulsions, either from physiological requirements or emulsifiability. Consequently, there is no valid reason for eliminating non-phosphatide emulsifiers from consideration in the development of fat emulsions.

An intensive investigation was begun into the emulsifying properties of various non-phosphatide emulsifiers which might be used for developing emulsions of the oil-in-water type with maximum physical stability. A simpler screening test was devised and employed to determine the relative emulsion stability imparted by 46 different emulsifiers in 96 systems.

The best emulsifier types were polyoxyethylene fatty alcohol, fatty ester of polyglycol, fatty amines, an acetylated ester of glycerol, and a fatty ester of polyglycerol. In general, best results with typical emulsions were obtained with combinations of these emulsifier types, dissolved in the oil phase rather than in the water phase.

About 10 combinations of the emulsifiers investigated warranted further study in emulsions prepared by high-pressure homogenization. The screening method was based on the assumption that if a system composed of an oil, emulsifiers, and water is agitated, the time required for separation of the oil and water phases should be an indication of the effectiveness of the emulsifier used. The results are, of course, relative and not absolute, though reproducible.

A typical example of the screening test used is the following: To 15 g. (30%) of oil in a beaker, 0.25 g. (0.5%) of an emulsifier was added and dissolved by gentle heating if necessary. Slow mechanical stirring of the oil phase was begun, as 35 g. of distilled water was added slowly. The speed of stirring was such as to avoid air entrainment. Other than that, stirring was not critical. The emulsion first formed generally was a thick cream, water-in-oil type, which reverted to the oil-in-water type with marked decrease in viscosity as water was added. About 30 seconds were allowed for the water addition, and the crude emulsion was stirred for one minute. The emulsion was then poured quickly into a 25-ml. graduate and at timed intervals demarcation of the phases was noted. Results were then calculated in terms of percentage of water phase separation as a function of time. Reproducibility of the results was of the order of 5%.

When the emulsifying system was composed of two or more emulsifiers, at least one was dissolved in the water phase.

Olive oil was used in these test emulsions. (At the time of these tests it was the preferred oil for intravenous fat emulsions.) Emulsifier concentration was 0.5%; in systems of multiple emulsifiers, 0.5% of each was used.

The emulsifiers which were screened, and the companies which supplied the samples, are listed in Table I.

TABLE I

EMULSIFIERS SCREENED FOR FURTHER TESTING.

Emulsifiers	Suppliers
Ethofats C/15, C/20, C/25 and 60/60	Armour Chemical Co.
Ethomeens C/20, C/25, 18/15, 18/25, and 18/60	Armour Chemical Co.
Ethomid HT/25	Armour Chemical Co.
Spans 20, 40, 60, 65, and 85	The Atlas Powder Co.
Tweens 20, 60, 65, 80, and 85	The Atlas Powder Co.
Brijs 30 and 35	The Atlas Powder Co.
G-931	The Atlas Powder Co.
Arlacel-C	The Atlas Powder Co.
G-7076H	The Atlas Powder Co.
G-672	The Atlas Powder Co.
MYRJ-51	The Atlas Powder Co
NNO	The Atlas Powder Co.
Methocel	Dow Chemical Co.
Drumulse	E. F. Drew & Co.
Polyglycol stearate 400	Glyco Products Co.
Aldo 25	Glyco Products Co.
Carbowax 4000	Glyco Products Co.
Carbopol 934	B. F. Goodrich
TEM	Hachmeister, Inc.
Kelcoloid LV	Kelco Co.
A-24	Rohm & Haas.

TABLE I (Cont'd.)

EMULSIFIERS SCREENED FOR FURTHER TESTING

Emulsifiers	Suppliers
Algin colloids	Seaplant Chemical Co.
Bone gelatin	Wilson & Co.
M. B. protein	Wilson & Co.
Pluronic F-68	Wyandotte Chemical Corp.
n-Dodecyl D-gluconamide	Northern Utilization Research Branch
n-Octadecyl D-gluconamide	Northern Utilization Research Branch
Polylactyl monostearin	Southern Utilization Research Branch
Palmitated dextran	Southern Utilization Research Branch
Dipalmitolactin	Southern Utilization Research Branch

On the basis of screening tests certain emulsifiers were selected for further investigation in emulsions prepared by high pressure homogenization. Those emulsifiers which were used in preparing emulsions by the latter method are given in detail in Section V.

Conclusions.

(1) Although a system of emulsifiers is generally better than a single emulsifier with respect to emulsion stability, a single emulsifier is more effective if present in the oil phase rather than in the water phase.

(2) Easier mixing of the oil and water phases is obtained by adding the water phase to the oil phase, not vice versa.

(3) A 30% concentration of oil results in a more stable emulsion than one of 10%.

(4) A lipophilic emulsifier in the oil phase and a strong hydrophilic-lipophilic emulsifier in the water phase gives the most stable emulsion.

C. PHYSICAL PROPERTIES

Solubility of Hydrophilic Emulsifiers. Oil-in-water emulsions for intravenous use must be heated to 121° C. for sterilization. Non-ionic emulsifiers, for example, those of the polyoxyethylene type, invert or become less soluble as the temperature increases, thus increasing the possibility of emulsion breakage. One of the factors which might influence the choice of emulsifiers for intravenous emulsions, therefore, is water phase solubility at elevated temperatures.

The solubility range of various emulsifiers was determined. The results show that the temperature at which solubility inversion occurs is highest in emulsifiers containing the maximum number of polyoxyethylene groups per molecule.

The usual oil-soluble emulsifying agents become more soluble in the oil with increases in temperature. Therefore, it was necessary only to investigate the solubility changes of the hydrophilic emulsifiers.

It is generally agreed that in stable O/W emulsions, the particles are spherical, close packed, and that the oil droplets are coated with monomolecular films which are liquid in nature. It has also been calculated that particles of 0.5 micron are most closely packed and result in maximum stability. Usually, the oil droplets are covered by one or more emulsifying agents, each of which has a hydrophobic and a hydrophilic end to its molecule. The former group is anchored in the oil droplet, and it is this group which forms the film on the oil particles. The hydrophobic (lipophilic) group is usually a long chain fatty acid group. The cross-sectional areas of such groups have been determined by many investigators by use of the Langmuir balance on monomolecular films.

In many water-soluble emulsifying agents the hydrophilic group is polyoxyethylenic. In such agents, the bonds between the water molecules and the emulsifying agent, which in turn links the oil to the water, are hydrogen bonds. The energy of dissociation of such a bond is of the order of magnitude of 7.0 kcal/mol. Simply, the kinetic energy of motion at the sterilization temperature is sufficient to break such bonds.

In conducting the experiments 1% solutions or dispersions of the various emulsifier types in water at room temperature were slowly heated until solubility inversion occurred. After inversion, the samples were allowed to cool and the temperature of clearing was observed. The warming procedure then was repeated. The inversion temperatures were found to be constant on repeated trials. The solubility characteristics of various emulsifier types are recorded in columns 3 and 4 of Table II. (Table II follows on page 41.) In addition to the polyoxyethylene type emulsifiers other types are also included for comparison. These latter emulsifiers have a limited solubility in water.

In order to determine whether the inversion temperature of emulsifiers in water solution was related to their behavior in actual emulsions with oil, several emulsions containing 15% by weight of a refined, bleached, and deodorized sesame oil and 1% of an emulsifier type (based on total weight) were made in a laboratory homogenizer. The emulsions were autoclaved in closed bottles with steam at 121° C. for 15 minutes. These results are also given in Table II.

The incorporation of a strongly lipophilic emulsifier in addition to a hydrophilic emulsifier would probably tend to prevent growth of particle size during homogenization and would also provide increased stability during autoclaving. Accordingly, a limited number of emulsions containing 15% of sesame oil were prepared with various lipophilic emulsifiers in combination with some of the water soluble emulsifiers containing in excess of 15 polyoxyethylene groups per alkyl group. Typical examples of such emulsions prepared by high pressure homogenization are given in Table III. (Table III follows on page 42)

It is recognized of course that many good emulsifiers are not completely soluble in water but form dispersions. Nevertheless, it is believed that a hydrophilic emulsifier should have sufficient affinity for water over the temperature range 5° - 121° to remain soluble.

As seen from Table I, with a given type of emulsifier containing polyoxyethylene groups the temperature at which solubility inversion occurs was found to increase as the number of polyoxyethylene groups increased. The variation in limit of solubility of the glycol ester type emulsifiers was from insoluble at room temperature for some, to soluble up to about 92° for others, as the number of polyoxyethylené groups per alkyn group increased from 5 to 50. The number of polyoxyethylene groups apparently is more important that the variation

The Water Solubility and Emulsification Characteristics of Various Emulsifiers

Type	POE groups[a/] per molecule	Inversion temperature °C	Appearance on cooling to 25° C	Emulsion description (Diameters of oil drops in microns) Before autoclaving[b/]	After autoclaving
POE glycol mono-esters					
Laurate(Etnofat C-15	5	-	dispersion	2-2.5, many 2.5-7.0, clusters	oil-water phases
Laurate(" C-20)	10	32	solution	2-3, many 7.0	oil-water phases
Laurate(" C-25)	15	66	solution	2-3, many 7.0	oil-drops-emulsion
Laurate(" C-60)	50	92	solution	0.7-1.5, some 4.0	oil-drops-emulsion
Stearate(" 60-25)	15	65	solution	0.7-1.5, some 5.0	oil-watery phases
Stearate(" 60-60)	50	98	solution	0.7-1.5, few 5.0	stable-0.7-1.4, few 5.0
Red Oil (" 142-15)	5	-	dispersion-oil layer	------	------
POE Amines					
Lauryl(Ethomeen C-15)	5	37	solution	0.7-1.5, many 2.0-4.0	5 mm oil layer-emulsion
Lauryl(" C-20)	10	98	solution	0.7-1.5, many 3.5	oil phase-watery phase
Stearyl(" 18-15)	5	-	gel	0.7-1.5, some 2-3, few 5.0	5 mm oil layer-emulsion
Stearyl(" 18-20)	10	74	dispersion	------	------
POE amides					
Lauryl(Ethomid HT-15)	5	-	dispersion	1-1.5, many 4-5.0	oil-water phases
Lauryl(Ethomid HT-25)	15	95	solution	1.0-1.5	5 mm oil layer-emulsion

POE Sorbitan mono-esters					
Laurate (Tween 20)	-	95	solution	0.7-1.5, many 2-3.5	oil drops-emulsion
Stearate(" 60)	-	76	solution	0.7-1.5, some 5.0	2 mm oil layer-emulsion
Oleate (" 80)	-	93	solution	0.7-1.5, some 4-5.0	small oil layer-emulsion
POE sorbitan polyesters					
Dilaurate (G7076H)	-	-	dispersion	0.7-1.5	2 mm oil layer-emulsion
Tristearate (Tween 85)	-	-	dispersion	-	-
Mixed triglyceride (G931)	-	-	dispersion	1.0 ------	stable - 1.0
POE alcohols					
Lauryl (Brij 30)	-	-	dispersion	0.7 - 1	1 mm oil layer
Lauryl (" 35)	(< C50)	100	solution	0.7-1,some 2.0	stable 0.7,some 2.0
Ethylene propylene oxide (Pluronic F68)	-	100	solution	0.7-1,some 2.0-3.0	oil-water phases
Sorbitan esters					
Monolaurate (Span 20)	-	-	gel	0.7-1.5, few 2-3.0	oil-water phases
Monopalmitate(" 40)	-	-	gel	0.7-1.5	oil-water phases
Monostearate (" 60)	-	-	gel	0.7-1.5	oil-water phases
Tristearate (" 65)	-	-	dispersion	-	-
Trioleate (" 85)	-	-	oil layer	-	-
Clycerol sorbitan laurate(G672)	-	-	dispersion	0.7-1, some 4.0	large oil drops-emulsion
Purified soybean phosphatides	-	-	dispersion	0.7-1, many 2.0	oil drops-emulsion
Polyglycerol oleic ester(Demal 14)	-	-	dispersion	2.0-3.0	oil-water phases
Polyethylene glycol stearate (PGS-400)	-	-	dispersion	0.7-1, some 2.0	1 mm oil layer
Monoglyceride (Myvacet)	-	-	dispersion	many 20-50	oil-water phases
Di-acetyl tartaric ester of mono- and diglyceride (TEM)	-	-	dispersion	1-2, many 3.0	oil layer-emulsion

a/ Polyoxyethylene
b/ Maximum temperature during homogenization 62°C

TABLE III

Emulsions With Two Emulsifiers

Emulsifiers	Concentration %	Stability to autoclaving
lauryl alcohol (Brij 30)	0.5 - 1.2	Unstable
yethylene-propylene oxide (Pluronic F68)	0.3 - 0.75	
er of mono- and diglyceride (TEM)	0.75 - 1.0	Unstable
yethylene-propylene oxide (Pluronic F68)	0.5 - 0.75	
cerol sorbitan triglyceride (G 931)	0.4 - 0.5	Unstable
yethylene-propylene oxide (Pluronic F68)	0.5 - 0.6	
lauryl alcohol (Brij 35)	0.45 - 0.75	Stable
cerol sorbitan triglyceride (G 931)	0.45 - 0.75	
glycol monoester (PGS-400)	0.3 - 1.2	Stable
ethylene-propylene oxide (Pluronic F68)	0.5 - 0.75	
etyl tartaric ester of mono- and glyceride (TEM)	0.3 - 0.5	Stable
ethylene-propylene oxide (Pluronic F68)	0.75	
erol sorbitan laurate (G 672)	0.2 - 0.8	Stable
ethylene-propylene oxide (Pluronic F68)	0.3 - 1.2	

Polyoxyethylene

in alkyl groups, as the laurcyl and stearoyl groups showed solubility inversion at temperatures of 66° - 68°, each having 15 polyoxyethylene groups per alkyl group. For the same number of polyoxyethylene groups per molecule, the amine type emulsifier was found to be more soluble than the amide, which in turn is more soluble than the fatty acid type.

Isoelectric Point. Because there may be some correlation between the stability of a phosphatide emulsion, its pH and the isoelectric point of the emulsifier, more information about the phosphatides used in emulsions was believed desirable. Studies are currently underway to measure the isoelectric point of such materials. A Northrup-Kunitz cataphoresis cell was assembled and calibrated, and measurements of the electrophoretic mobility of particles, observed through a microscope, in a buffered dispersion of purified egg lecithin were made over a pH range from 3.7 to 8.0. Using egg lecithin purified by chromatographing on alumina (and stored under nitrogen in the deep-freeze at -25°C.) a reversal of mobility was noted with the particles behaving as positive ions at the lower pH values and as negative ions at the higher pH values. The preliminary measurements indicated the point of mobility reversibility of this egg yolk lecithin to be not lower than about 5.6 pH. However it appeared that the direction of particle mobility seemed to change (equivalent to lowering of the isoelectric point) with duration of the tests as would be expected if decomposition was occurring. This work is being continued.

Lecithin Dispersions. Water dispersions of three lecithins were stored at 45°C. to observe stability and change in pH. The three lecithins were purified egg lecithin, lecithin used by The Upjohn Co., and crude egg lecithin. The dispersions (1.2% in water) were made in a small Virtis laboratory homogenizer in which the particle size was reduced sufficiently for satisfactory observation of the stability of the dispersions. Dispersions of Upjohn lecithin in concentrations of 2.4% and 4.8% in water were also prepared. Observations of pH, particle size and color were made from time to time over a period of four months. These results may be summarized as follows:

The purified egg lecithin dispersion had the highest pH while the crude egg lecithin had the lowest pH, with the Upjohn lecithin intermediate in pH. This pattern existed throughout the tests. After 4 months' time all of the 1.2% dispersions were fairly good although there were increases in particle size, some darkening in color and some indication of a coagulant or sediment. The dispersions became more acidic, having fallen to a pH range of 3-3.4. Titration of samples of the purified egg lecithin dispersion indicated about 0.6% free fatty acid (as oleic acid) at about two months' standing and about 0.9% F.F.A. after four months time. The higher concentrations of lecithin dispersions were completely broken after 3 months.

A sample of purified egg lecithin was also stored dry at 45°C. Although this sample was white to pale yellow in color when initially prepared and was stored under vacuum over P_2O_5 at about 0°C before use, it had a very light tan color when these tests were started. After three months it had turned to a dark brown color and developed a very strong amine-like odor.

Conclusions. The bonds between some water soluble emulsifiers and water are hydrogen bonds with low energies of dissociation, of the order of 7 kcal./mole. In the absence of other factors, such bonds can be broken by the kinetic energy of motion at elevated temperatures.

The water solubility of many emulsifiers is imparted by their content of polyoxyethylene groups. Those emulsifiers which contain 15 or less such groups per molecule undergo inverse solubility below 68°C.; those with 50 or more such groups are completely soluble up to a limit of slightly above 100°C.

Solubility inversion of polyoxyethylene containing emulsifiers is dependent to a greater extent on the number of polyoxyethylene groups than to variation of the alkyl groups commonly present. The temperature at which solubility inversion occurs increases as the number of polyoxyethylene groups increases. Polyethylene-propylene oxide had the longest solubility range of the emulsifiers tested.

An increase in particle size and in some cases phase separation of emulsions prepared with emulsifiers which undergo solubility inversion below 85° was found. Emulsions prepared with emulsifiers whose inversion temperatures were above 85° maintained, generally, a small particle size on autoclaving.

Emulsions prepared with two emulsifiers, such that one had some lipophilic characteristics stronger than the other, were found to be stable and maintain a small particle size on autoclaving.

Water dispersions of lecithin samples indicated the probability of decomposition of all the lecithins during storage.

The lecithins, particularly in the pure state, are hygroscopic and sensitive to light, air, and heat. This instability is evident even when stored in closed containers at room temperature.

V. NON-PHOSPHATIDE EMULSIONS

The non-phosphatide emulsifiers which are listed in Section IV. have been used in preparing approximately 400 emulsions by high pressure homogenization. Many of these emulsions are as physically stable as phosphatide-containing emulsions. Some have been found non-toxic to rats. A discussion of these emulsions follows, including preparation, toxicity and stability, and current status.

A. Preparation

The results of the screening test employed offer some indication as to which emulsifier systems should give the best emulsions. Those systems which gave the most stable emulsions in the screening test were therefore used in preparing emulsions by high pressure homogenization. Factors which were determined in preparing emulsions by homogenization included pressure of homogenization and the number of cycles required to produce satisfactory particle size. Of the emulsions prepared thus far, those containing Drumulse as part of the emulsifier system were extremely easy to homogenize.

Pressure of Homogenization. One of the factors investigated early in this program was the effect of homogenization pressure on the resultant size of the dispersed oil droplets in an emulsion. The homogenizer used was a Cherry Burrell Superhomo, capable of operation at any pressure up to 5000 psi. The test emulsion contained 30% olive oil (this work was done before it was agreed to limit experiments to cottonseed oil) in which was dissolved 1% Span 60. This oil phase was put into the supply tank of the homogenizer and continuously cycled at 2000 psi. The water phase was slowly added, resulting in a thick water-in-oil preliminary emulsion. As the water phase continued to be added, the emulsion reverted to the oil-in-water type. On addition of the entire water phase, the emulsion was cycled batchwise through the homogenizer at the same operating pressure for 8 complete cycles. Particle size was determined, the pressure then increased to 2500 psi, and the procedure repeated. Pressure was increased in increments of 500 psi. to a final pressure of 4000 psi. An emulsion temperature of 45°C was maintained throughout. Particle size was determined microscopically using an oil immersion lens at a magnification of 950. The results are given in Table I. (Table I follows on page 46.

With the particular homogenizer used and with Span 60 as the emulsifier, 3500 psi was required to obtain a uniform particle size of 0.7 to 1.0 microns. Below this pressure some particles were of this size, but particles generally were larger.

In all subsequent emulsion preparations, 3500 psi. was the standard operating pressure.

TABLE I.

Effect of Pressure on Particle Size of Homogenized Fat Emulsions

Pressure, psi.	Cycles	Particle size, microns
2000	8	Most particles 1.5-2.0
2500	8	Most particles less than 1.5-2.0
3000	8	Most particles less than 1.5-2.0
3500	8	Largest particle 0.7, most $<$ 0.5

In subsequent work with emulsions of 15% oil content it was found that the order of addition of phases was not critical there being no difference in resulting particle size whether the oil phase or water phase was added first. Therefore to avoid the formation of water-in-oil emulsions, the water phase w as added first.

Currently, emulsions are prepared by homogenization in the following manner. The lipophilic emulsifier(s) is dissolved in the oil phase with gentle heating if necessary. The hydrophilic emulsifier is dissolved in about 75% of the required amount of 5% dextrose solution, and this is put into the homogenizer supply tank. Continuous cycling of this phase is begun, and the operating pressure of 3500 psi obtained. The oil phase containing the dissolved emulsifier is slowly added. The crude emulsion is cycled batchwise twice, and the remaining dextrose solution added. After two additional cycles, the emulsion is examined microscopically for particle size, and if satisfactory is pumped from the homogenizer. The bottled and sealed emulsion is then autoclaved at 121°C. for 15 minutes.

With Drumulse in the emulsifying system, only two complete homogenization cycles are required.

B. Toxicity and Stability

Approximately 400 non-phosphatide emulsions have been prepared by high pressure homogenization, using those emulsifiers which appeared to be most suitable on the basis of the screening test (See Section IV, B). Of this number, 14 possessed a sufficient degree of physical stability to warrant a preliminary determination of their toxicity to rats. The Army Medical and Nutrition Laboratory found that of the 14 emulsions submitted thus far, 5 were non-toxic to rats at the 40 ml./kg. level.

These emulsions were prepared in the same manner previously described. They were emulsified in a Cherry-Burrell Superhomo, and autoclaved at 121°C. for 15 minutes. Each contained 15% cottonseed oil (specially selected "Wesson" brand) and 5% dextrose solution. The emulsifiers and their concentrations are listed in Table II. (Table II follows on page).

There are no animal testing facilities available at the Southern Regional Laboratory. Arrangements were made with the Army Medical and Nutrition Laboratory, Fitzsimons Army Hospital, Denver, Colorado, whereby the emulsions would be tested, in a preliminary manner, for toxicity to rats. The test consisted of injection of the test emulsions at several dosage levels, and observation of gross effects. The emulsions which have been tested and the results found are given in Table II. Emulsifier concentrations are given as percent of weight of entire emulsion. The emulsifiers Carbopol, Pluronic F68, Brij 35, and Ethomeen 18/25, where used, were dissolved in the dextrose solution (water phase); other emulsifiers in the oil phase.

TABLE II

Results of Rat Toxicity Tests of Emulsions With Various Emulsifier Systems

ılsion No. and ılsifiers (%)	Dosage ml./kg.	Rats tested	Deaths	Remarks
	40			
71-15W		4	4	Multiple white spots over entire
I (1.0)				surface of liver.
bopol (0.1)	20	4	2	Marked hematuria.
127-15W	40	4	4	
I (0.5)	20	3	1	Died within 48 hours.
bopol (0.1)				No hemoglobinuria.
ronic F68 (0.7)				
183-15W	40	4	4	Longest survival 12 minutes.
ohn lecithin (1.2)				
bopol (0.1)				
ronic F68 (0.3)				
184-15W	40	4	1	No hemoglobinuria. Died in
ohn lecithin (1.0)				8 minutes.
j 35 (0.5)				
185-15W	40	4	0	No hemoglobinuria.
(0.5)				
ohn lecithin (0.7)				
ronic F68 (0.7)				
193-15W	40	5	0	
(0.5)				
[illegible] (0.5)				

Milk	Protein-rich Foods	Vegetables and Fruits			Bread, Enriched or Whole-grain	Butter or Fortified Margarine	Additional Foods to Meet Energy Needs of Children
Serve 1/2 pint daily	Serve 2 ounces or equivalent daily (see individual items listed below)	Serve 3/4 cup vegetables or fruits or a combination of both daily			Serve 1 slice or equivalent daily	Serve 2 teaspoons daily	Serve as needed
Serve as a beverage	Serve as main dish (plain or in casserole, stews, loaves, etc.) or in soups, salads, and sandwiches	Serve vegetables - Raw, steamed, boiled, or baked, alone or in salads, casseroles, and stews Serve fruits - Raw or cooked, alone or in fruit cups, salads, or desserts			Serve buttered or in sandwiches	Serve as spread for bread or as seasoning	Serve as extras or larger initial servings, second servings of main dishes, sandwiches, and salads
Fresh, fluid whole milk	**Cheese** 2 oz. Cheddar Cottage **Dry Beans and Peas** 1/2 cup cooked **Eggs** Dried, whole 2 T (dry) Frozen, whole 3-1/4 T Shell 1 **Fish**, as served without bones 2 oz. Canned Fresh and frozen fillets **Meat**, lean as served, without bone 2 oz. (bacon and salt pork do not qualify) Beef Lamb Pork Veal Variety Meats Heart Liver* Tongue Frankfurters Lunchmeats **Peanut Butter** 4 T. **Poultry**, as served, without bones 2 oz. Chicken Turkey * Also good source of vitamin A. ** Also good source of vitamin C.	**Vitamin A Foods** **Serve twice a week** **Good Sources** FRUITS **Deep Yellow** Apricots Cantaloup** Mango** Persimmons VEGETABLES **Dark Green, Leafy** Beet greens Broccoli** Chard Collards** Cress* Dandelion greens Kale** Mustard greens** Spinach** Turnip greens** **Deep Yellow** Carrots Pumpkin Squash, winter Sweetpotatoes** **Fair Sources** FRUITS Cherries Papaya Peaches Prunes, dried Tangerines Tangerine juice Tomatoes, juice, paste, puree Watermelon VEGETABLES Asparagus, green Peas, green, immature	**Vitamin C Foods** **Serve every day** **Good Sources** FRUITS **Citrus** Grapefruit Grapefruit juice Orange Orange juice Tangerine Tangerine juice **Other** Cantaloup* Gooseberries Guavas Honeydew melon Mango* Papaya Strawberries Tomatoes VEGETABLES Asparagus, green Broccoli* Brussels sprouts Cabbage Celery or Chinese cabbage Collards* Cress* Kale* Kohlrabi Mustard greens* Pepper, green Spinach* Sweetpotatoes* Turnip greens* **Fair Sources** FRUITS Blackberries Blueberries Pineapple Raspberries Tomato juice, paste, puree VEGETABLES Cauliflower Cowpeas, immature seed Dandelion greens Potatoes (other than mashed or fried) Rutabaga Sauerkraut	**Other Foods** **Serve as needed** FRUITS Apples Applesauce Avocados Bananas Cranberries Figs Fruit cocktail Pears Plums Raisins Rhubarb Fruit juices (apple, grape, pineapple etc.) VEGETABLES Beans, lima, green Beans, snap Beets Celery Corn Cucumbers Eggplant Lettuce Okra Onions Parsnips Potatoes, mashed or fried Squash, summer Turnips	**Loaf** Raisin Rye Soy White Whole wheat **Other** Biscuits Brown bread Cornbread Muffins Rolls	Butter Margarine, fortified with 15,000 I.U. vitamin A	**Cereal and Other Grain Products** Crackers Macaroni Hominy Noodles Rice Spaghetti **Desserts** Cakes Cobblers Cookies Ice cream Pies Pudding **Other** Honey Jams Jellies Molasses Nuts Sirup

Emulsion 71-15 W had exceptional physical stability, but was found to be toxic. In attempting to determine the cause of this toxicity, it was found that this emulsion was clustered by human albumin in vitro. Pluronic F68 was found to prevent the clustering, and was incorporated into emulsion 127-15W. together with TEM and Carbopol. However, 127-15W also was toxic. Emulsions 183-15W and 185-15W therefore were designed as an attempt to determine whether either or both TEM or Carbopol were toxic. Upjohn lecithin and Pluronic F68 have been used as non-toxic emulsifiers, and were therefore used to prepare emulsions in which were included, individually, Carbopol and TEM. Results indicate TEM to be non-toxic.

In like manner, emulsion 184-15W was used to determine the toxicity of Brij 35.

Emulsion 382-15W contained a synthetic emulsifier, F-10, which was prepared and furnished by Dr. Louis F. Fieser of Harvard University.

Stability. One of the properties which an emulsion should possess is ability to withstand prolonged storage--i.e., physical stability and resistance to mechanical shock. Determination of this stability is most conveniently carried out by means of an accelerated test.

There are several such tests in use, the most common being variations of a shaking technique. Two of the variables in this test, namely position of the bottle and amount of emulsion contained therein during the course of shaking were investigated and standardized.

The mechanical shaker used consisted of a horizontal platform upon which sample bottles could be clamped. The platform was then made to move forward and back in a horizontal plane at about 140 cycles per minute. Sample bottles were 4 ounce capacity, approximately 1 inch in diameter and 6 inches tall.

The amount of emulsion contained by the sample bottles was found to be an important variable in the shaking test. Portions of the same emulsion in the amounts of 25, 50, and 100 ml. were shaken together, and it was found that growth in particle size to 7 microns was fastest in the 50 ml. portion of sample. This quantity is the amount now used in all shaking tests.

Emulsions which are considered to be physically stable are those in which there are no particles of 7 microns or more in diameter after a 1 hour shaking by the above procedure. This is of course an arbitrary basis. A phosphatide emulsion is able to withstand 2 hours of shaking. All of the emulsions listed in Table II were considered stable. Emulsions 71-15W, 222-15W, 318-15W, and 382-15W were shaken for 3 hours before particles over 7 microns could be observed.

C. Status

The preparation of non-phosphatide fat emulsions has reached a point at which such emulsions are equal or superior to phosphatide-containing emulsions in physical properties. Many emulsions prepared at the Southern Regional Laboratory have had physical characteristics comparable to phosphatide emulsions, but were toxic to rats. The current emulsions are not only physically stable, but on the basis of preliminary tests are non-toxic to rats.

The best emulsion from all aspects contains the emulsifiers TEM, Drumulse, and Pluronic F68. Two different formulations of these ingredients have been found non-toxic to rats when injected at the dosage of 40 ml./kg. The physical stability is very good, and ease of emulsification excellent. This emulsion is now ready for more intensive animal testing and arrangements are being made to secure further cooperation in its evaluation.

VI. PHYSICAL EVALUATION OF EMULSIONS

Emulsions in which soybean lecithin is part of the emulsifying system currently are used in clinical investigations, not, however, without variable physiological effects. A chemical and physical evaluation of such emulsions therefore has been underway for some time to determine, if possible, the cause of the adverse effects. This investigation includes the browning which occurs in phosphatide-containing emulsions; chromatographic analysis of phosphatides; the particle size distribution in emulsions; irradiation of emulsions as a possible means of sterilization; and the electrophoretic mobility of emulsions.

Progress has been made especially in characterizing the nature of the brown material formed in phosphatide containing emulsions, in chromatographic analysis of pure phosphatides and their hydrolytic cleavage products, and in determining the effect of emulsions on the electrophoretic patterns of blood serum.

A. BROWNING

Phosphatide-containing fat emulsions have been observed to develop a colloidal dispersion of a brownish material, which does not develop in emulsions which are made with non-phosphatides. It appeared that the phosphatides could be reacting with the glucose (present in emulsions) to form the brown material. The brown color *per se* is not objectionable but since the brown material is solid in nature it could be responsible for the occasional adverse physiological reactions when emulsions containing soybean phosphatides are administered clinically.

Chromatograms of the reaction products derived from dextrose and lecithin and from dextrose and cephalin were obtained and compared with chromatograms of the brown material isolated from emulsions. Evidence was obtained which indicated that the brown material results from reactions between dextrose and soybean phosphatides.

Several batches of phosphatide-containing emulsions were prepared. Brown material was collected from these emulsions and also from emulsions prepared by others (The Upjohn Co. and Harvard University). The brownish material was colloidal in nature, and was therefore separated only with difficulty from the emulsions. Repeated freezing and high speed centrifugation were necessary before the brown colloidal dispersion could be flocculated from the emulsion. Occasionally, a "bad" batch of phosphatide-containing emulsion produced an observable precipitate on shelf storage. In most cases, the brown colloidal material was not detectable in the freshly prepared emulsions. However, it could always be separated once the colloidal dispersion was broken.

Since this brown material was observed only in phosphatide-containing emulsions, it appeared likely that the brown material contained a cephalin or lecithin fraction of the soybean phosphatides as one of its components. Therefore, the isolated brown material was chromatographed by the method of Hanahan (1) and by a procedure similar to that used on cerebrosides (2).

The chromatograms of the brownish material were compared with the chromatograms of a "pure" yeast lecithin, choline chloride, dextrose, animal phosphatides and soybean phosphatides. Chromatograms of these same substances in 5% dextrose solutions before and after autoclaving were also made and compared with the chromatograms of the brown material. A chromatographic technique using glass fiber strips impregnated with silicic acid was used, as it gave rapid separation of the cephalin and lecithin portions of phosphatides. These results are given in Table I (follows on page 53). A more detailed discussion of the results of this series of experiments is covered in a manuscript (3).

For determining the toxicity of the brown material from phosphatide-containing emulsions, an emulsion was made and to it was added some isolated brown material. The emulsion contained 1.2% purified soybean phosphatide, 0.3% Pluronic F68, 15% cottonseed oil and dextrose solution (5%). Brown material which had been centrifuged from another phosphatide emulsion was added in the amount of 2%. An identical emulsion, without added brown material, served as control. Results of the testing, by the Medical and Nutrition Laboratory, Fitzsimmons Army Hospital, are:

Dose ml/kg	No. of deaths among 5 rats control	with brown material
40	0	0
48	1	1
58	1	1
69	1	0
83	-	4

On the basis of these tests, it is concluded that there is no significant difference in acute toxicity of the two emulsions. No tests were made to determine thermogenic effects of the brown material.

Conclusions. The R_f values of the reactive spots on chromatograms of reaction products of various phosphatides and choline chloride with dextrose are similar to those from chromatograms of the brown material isolated from emulsions. This would indicate that the brown material probably results from a reaction of soybean phosphatides with dextrose. There are some indications that even the lecithin fraction participates in "Browning-type" reactions.

B. PAPER CHROMATOGRAPHY

A method for determining the purity of lecithin preparations and detecting possible cleavage products of phosphatides on storage or on incorporation into an emulsion is needed. Various phosphatides were therefore characterized by the cellulose paper chromatographic method of Hanahan (1) and the silicic acid impregnated glass fiber method of Dieckert (2).

TABLE I

R_f Values of the Cephalin and Lecithin Fractions Obtained by Chromatography

Materials	R_f Values	
	Choline (Lecithin Fraction)	Ninhydrin Positive Groups (Cephalin Fraction
"Pure" Yeast Lecithin		
Ether solution	0.07 0.31	
Vater dispersion	0.07 0.30	
)ispersion in 5% dextrose solution	0.05 0.29 0.55	0.61
)ispersion in 5% dextrose solution after autoclaving	0.09 0.31 0.55	0.91
ater dispersion of product autoclaved dry with dextrose	0.05 0.28	0.92
Choline Chloride		
extrose solution	0.24 0.45	0.71
itoclaved dextrose solution	0.44	0.72
iter dispersion of product autoclaved dry with dextrose	0.24	0.73
Soybean Phosphatides*		
her solution	0.25	0.05 0.81
ter dispersion	0.44 0.80*	*0.04 0.74
toclaved water dispersion	0.43 0.77	0.04 0.74
spersion in 5% dextrose solution	0.28 0.44 0.77	0.04 0.80

TABLE I Continued

Autoclaved dispersion in 5% dextrose		0.29**	0.44	0.75	0.04	0.75	
Water dispersion of product autoclaved dry with dextrose			0.50			0.72	
Autoclaved dry		0.28	0.56		0.04	0.73	
Animal Phosphatides* **							
Ether solution	0.03	0.26			0.05	0.74	0.82
Water dispersion	0.03	0.26			0.04	0.73	0.82
Autoclaved water dispersion	0.03	0.26			0.04	0.73	0.82
Dispersion in 5% dextrose solution	0.05		0.72		0.06		0.79
Autoclaved dispersion in 5% dextrose solution	0.04		0.73		0.04	0.70	
Water dispersion of product autoclaved dry with dextrose		0.29**	0.44	0.75	0.04	0.75	
Brown Material Isolated From Emulsions	0.03	0.26	0.52	0.80	0.03	0.76	

* Contains naturally occurring sugars

** Weak spot

** Small amount of lecithin

Using the glass fiber paper, a new solvent system was devised which affects the separation of compounds that move together in the Hanahan system and which permits the detection of smaller quantities of material.

Soybean phosphatide and egg lecithin emulsions chromatographed on either cellulose paper or glass fiber paper showed significantly different patterns than when the phosphatides were chromatographed alone indicating that changes had occurred. The nature of these changes is being further investigated.

Descending paper chromatograms were run at room temperature on Whatmann No. 1 paper in glass cylinders. A water-saturated butanol solvent system was used in all of the runs. The papers, after application of the samples, were typically equilibrated overnight and developed for 7 hours or equilibrated for 24 hours and developed overnight. Very short equilibration periods (less than an hour) were also tried. Variations in equilibration and development time did not significantly affect R_f values.

Spots were detected on the chromatograms by the following reagents:

Reagent	Group tested for
Molybdate - perchloric acid	Organic phosphate
phosphomolybdic acid - $SnCl_2$	choline
potassium permanganate	unsaturation
ninhydrin	primary or secondary amine
bromcresol green	free fatty acid
periodate - starch	vicinal glycol

R_f values of various phosphatides and other pertinent materials are given in Table II (table follows on page 55).

On applying this technique to phosphatide emulsions, it was found that the phosphatide portion of the emulsion behaved quite differently from the corresponding phosphatide alone. Soybean phosphatide emulsion and egg lecithin emulsion (both before and after autoclaving) gave a strongly positive choline test near the origin of the chromatogram (R_f 0.0 streaked to approximately 0.15). This slow-moving material was not found on chromatograms of the phosphatides alone or of the phosphatides in the presence of fat. Also, excessive quantities of emulsion had to be applied to the paper before phosphatide tests were obtained at the position characteristic of lecithin and cephalin (R_f 0.84).

Glass fiber chromatograms of the soybean phosphatide emulsion run in a methanol-ether (1:1) solvent also showed the presence of this unidentified quarternary ammonium compound streaked from the origin. It was not detected by spraying the chromatogram with concentrated sulfuric acid and charring over a hot plate.

TABLE II

TABLE II

R_f Values* of Several Phosphatides and Other Pertinent Materials

Sample	Primary Amino Group	Choline	Unsaturation	Phosphate	Free Fatty Acid	Vicinal Glycol
Crude egg lecithin	.83 and .00 to .06	.83 and .00	.85	.81		
Pure egg lecithin		.83	.83	.82		
Upjohn "lecithin"	.80 and .00 to .03	.81 and .00	.84 and .00	.82 and .00		
Cottonseed lecithin	negative	.85	.83	.85	negative	
Hanahan yeast lecithin		.85	.84	.85 and .00		
Choline chloride		.08				
Glucose			.07			.07
Monopalmitin						.93
Linoleic acid			.92		.95	
Linolenic acid			.96		.96	
Stearic acid					.93	
Palmitic acid					.91	

* These are average values obtained from several chromatograms on cellulose paper.

A phenol solvent system was devised for use with the silicic acid impregnated glass fiber paper which effectively separates lysolecithin sphingomyelin, lecithin, cephalin and fatty acids. The spots may be detected on the chromatogram with the usual spray reagents or by charring with sulfuric acid. Choline chloride, ethanol amine, sodium glycerophosphate, fatty acids, and glycerol have also been characterized by this system.

Chromatograms of "pure" egg lecithin (fractionated on an alumina column) run in the phenol system showed the presence of two choline-positive impurities in the sample. These contaminants had not been detected on cellulose paper. The R_f values of these additional spots correspond to those of lysolecithin and sphingomyelin.

C. PARTICLE SIZE DISTRIBUTION

At the February 1955 meeting of the OSG Task Force at the Southern Regional Research Laboratory it was suggested that the presence of submicroscopic particles (less than 0.5 micron in diameter) in the emulsions for intravenous alimentation might be responsible for the severe back pains experienced by some patients receiving the emulsions. A literature search revealed no methods to determine size distributions in o/w emulsions containing particles less than 0.5 micron in diameter and of low molecular weight. Several methods usually applied to the determination of the size of colloidal particles of less than 0.5 micron diameter were applied to the o/w emulsions, including methods using the ultramicroscope zone electrophoresis, and ultracentrifugation techniques. Although none of these methods have proven satisfactory, the most promising appears to be zone electrophoresis.

The ultramicroscope. The instrument used was an early modification of the slit type made by Bausch and Lomb. The light source was a carbon arc. Magnifications up to approximately 10,000 diameters were employed on emulsions of various dilutions. Very little scattered light was observed, and no well defined patterns were obtained. It was concluded that because of the small difference in the refractive indices of the dispersed phase and the dispersing medium, the oil droplets could not be observed with this particular ultramicroscope.

Zone Electrophoresis. At present this method appears to be most promising for determining particle size distribution. A small amount of an o/w emulsion, containing soybean phosphatide and Pluronic F-68 as emulsifiers, was applied to a filter paper strip soaked in buffer at pH 8.6, and an electric current applied. This method was unsuccessful when filter paper was used as the supporting medium, but when glass fiber filter paper was substituted for the cellulose paper, the emulsion separated into two fractions. High speed centrifugation at 11,000 rpm prior to electrophoresis (previous centrifugation and microscopic examination of the centrifuged emulsion proved centrifugation did not alter the size of the particles) resulted in the separation of two fractions. No absolute sizes

of particles have been determined, but the oil droplets in the lower layer of the centrifuged emulsion had electrophoretic mobilities 2.8 times greater than the largest oil droplets present in the upper layer. The upper layer contained the smaller particles in addition to the few larger particles. Microscopic examination of the lower layer revealed only a few particles, but all were 0.5 - 0.7 μ in diameter. Microscopic examination of the upper layer showed it to be more concentrated with respect to oil particles. The majority of particles were still 0.5 - 0.7 μ in diameter, but there were also present a few particles which were 1.5 - 2.0 μ in diameter. These larger particles moved only 1/3 as fast as the smaller particles.

Absorbency curves in the visible range have been determined on dilutions of the whole emulsion and of the upper and lower layers after centrifugation at various speeds and for various time intervals. These are preliminary experiments, and are to be followed by light scattering experiments in the shorter wave length range. A correlation of light scattering data with electrophoretic data may give a determination of the quantity of oil in the smallest droplets and an estimation of the absolute size of these droplets.

Ultracentrifugation. Preliminary experiments on the sedimentation equilibria of the fat emulsions in the ultracentrifuge have been initiated. Early experiments indicate that the particles are approximately 0.5 μ in diameter. At present, we have no data giving evidence for the existence of submicroscopic particles in our emulsions.

Other Methods. It was suggested that the electron microscope could be used to determine the particle size distribution in the emulsions, if a replica of the particles could be made. If the emulsion could be rapidly frozen to extremely low temperatures and if there were no distortion or change in size of the oil droplets, the surface of the frozen emulsion might then be carefully shaved off, leaving exposed oil droplets. The oil would be removed with a solvent and a replica made of the indentations left by the oil droplets. Preliminary observations under an ordinary microscope of an emulsion as it was frozen using an acetone and dry ice bath on a cold stage microscope revealed that by this method of freezing, the emulsion was broken. This method was not pursued any further.

On the assumption that the molar volume of the triglyceride molecule within the oil droplet is the same as it is in a liquid oil, and that the surface and length of the molecule of the emulsifier at the oil/water interface are the same on the spherical droplets as in monomolecular films at oil/water interfaces, the number of the oil molecules per oil droplet of different diameters was calculated. From these calculations, it was concluded that it is very unlikely that droplets of less than 0.1 micron in diameter would exist, but that particles of 0.5 - .7 μ in diameter could accommodate the triglyceride molecules within the oil droplet and the emulsifier layer surrounding the oil droplets.

At present, it is concluded that an emulsion of the Upjohn type has a general particle size of 0.5-0.7 m. in diameter. There are occasional droplets as large as 1.5-2m.in diameter. The majority of particles (0.5-.7m.) in diameter have an electrophoretic mobility of 1.2×10^{-5} cm/sec per volt/cm under our experimental conditions, and the very few larger droplets (1.5-2 m. in diameter) have an electrophoretic mobility of 0.4×10^{-5} cm/sec. per volt/cm. We have no experimental evidence for the existence of submicroscopic particles. The absolute sizes of the oil droplets are not known at present, and work will be continued in an attempt to calculate the absolute sizes of the oil droplets from data made available by use of the ultracentrifuge and light scattering experiments. This work may result in the determination of the size distribution of the particles within an emulsion.

D. STERILIZATION BY IRRADIATION

The use of irradiation as a possible means of sterilizing fat emulsions has undergone limited investigation. Fat emulsions prepared by the Southern Regional Research Laboratory were irradiated by the Quartermaster Food and Container Institute. The two main objectives are to determine (1) does irradiation alter the physical, chemical, or physiological characteristics of a fat emulsion, and (2) does irradiation sterilize a fat emulsion as effectively as does steam autoclaving.

Preliminary data on the first phase of this program has been obtained. An emulsion was prepared with 15% cottonseed oil, 1.2% phosphatide (Upjohn sample), and 0.3% Pluronic F68. After steam autoclaving, several bottles of this emulsion were shipped to the irradiation facilities of the QMFCI and irradiated at three million rep. The irradiated emulsions on their return showed little or no physical damage, although their stability to shaking was somewhat less than the control. A penetrating grassy odor and flavor were the most obvious changes. Physiologically there was no difference in the toxicity to rats of the irradiated and control samples as determined by the Army Medical and Nutrition Laboratory, Fitzsimons Army Hospital.

Rat Toxicity of Irradiated and Non-Irradiated Emulsions

Emulsion	Dosage	Rats tested	Deaths	Remarks
	ml/log			
Irradiated	40	5	0	
	80	5	2	One died immediately, another 5 days later.
Non-irradiated	40	5	0	

In thermogenic studies, the changes in temperature of 4 rabbits given non-irradiated emulsion were: +0.9, -1.2, +0.3, +0.3 and irradiated emulsion: 0.0, +1.4, +1.4, +0.9.

Further investigation into the irradiation of fat emulsions is dependent upon a need for such a procedure. At present, both phosphatide and non-phosphatide emulsions can be prepared and successfully autoclaved.

E. ELECTROPHORESIS

Several oil in water (o/w) emulsions have been prepared which were stable to autoclaving and to mechanical shock. As far as these physical tests were concerned, these emulsions were "stable" o/w emulsions. However, it was observed that certain apparently stable emulsions were broken when brought into contact with human serum. In an attempt to correlate the stability of fat emulsions in the presence of human serum with rat toxicity data, additional in vitro tests were introduced which included observations on the stability of emulsions in the presence of isotonic sodium chloride and polysol solutions, 4% aqueous solutions of human serum albumin, and the above-mentioned pooled human serum. The object of these investigations was to determine whether the actual clustering of the emulsion on coming into contact with the serum proteins could contribute to hematuria and other adverse physiological effects, or if the emulsifiers per se were toxic.

As a result of a series of experiments on serum-emulsion mixtures, it was found that there were only a few emulsifiers which were able to protect the oil droplets from the serum proteins. It was also observed that a few emulsions were stable in human serum but not in rat serum. Furthermore, some of the emulsions which were very toxic to rats were extensively clustered by rat serum.

In order to get more fundamental data on the interaction between fats or emulsifiers and the serum proteins, an investigation of the changes in the electophoretic patterns of the serum proteins in the presence of various fat emulsions was initiated. Very definite changes in the mobilities and amounts of protein fractions of serum when mixed in vitro with certain fat emulsions have been observed. The fat furnished by non-phosphatide emulsions had electrophoretic mobilities closer to that of the naturally occurring fat in the normal serum. By this method of investigation in vitro and by following the blood patterns in dogs given emulsions it should be possible to select an emulsion which will furnish a fat most nearly like the chylomicra and which will not alter the electrophoretic pattern of the serum proteins.

Electrophoreograms. Electrophoreograms of serum were made according to a standardized procedure which gave the best protein fractionation. Ten or 20 microliters of serum were applied at the mid-point of horizontal strips of Whatman 3MM paper after the strips had been equilibrated in the electrophoresis cabinet. Veronal buffer (5,5-diethylbarbituric acid) of pH 8.6, ionic strength 0.05, and an atmosphere of helium gas were used in the cabinet.

The voltage was applied after putting the sample on the strips, and the amperage (approximately 3.5 ma) as well as the voltage (350 v) was found to be constant over 16 hour intervals. Under these conditions, reproducible fractionation patterns were obtained, and the mobilities of the various fractions of pooled normal serum were found to be the same in duplicate determinations. The strips were dried in air, and then in an oven at 100° C. for 20 minutes. Strips were stained individually for proteins by use of a 0.1% solution of bromphenol blue in 95% ethanol saturated with $HgCl_2$ and.5% with respect to acetic acid, and for fats by use of a saturated solution of Oil Red O in 60% ethanol.

Similar experiments with sera on glass fiber strips were performed, but the fractionations were not as good and some of the stained fractions washed off the glass fiber more easily during the staining processes.

Electrophoreograms of whole emulsions, and the top and bottom layers of emulsions centrifuged at 11,000 rpm for 20 minutes were made. In these experiments, glass fiber strips were used, since migration was not observed on cellulose strips, but was observed on glass fiber strips under the same conditions. These strips were stained with Oil Red O.

Some of the data were obtained on 1:1 mixtures of emulsions and serum, but it was decided later to lower the emulsion:serum ratio to 0.05:1 in order more nearly to approach the fat:serum ratio found in normal sera after fatty meals. Mixtures of serum and various emulsions, at the volume ratios emulsion:serum of 1:1 and 0.05:1, were applied to paper strips and their electrophoreograms compared with those of sera alone under the same conditions. All emulsions used contained 15% by weight of cottonseed oil and 5% dextrose, but different emulsifiers. The emulsions were those for which rat toxicity data and data on stability to human sera were known.

A few mixtures of rat serum and emulsions at volume ratio of emulsion: serum 0.05:1 were applied to paper strips, and their electrophoreograms compared to those of rat serum alone under the same conditions.

The effect of adding a phosphatide and a non-phosphatide emulsion to dog serum in vivo and in vitro was investigated by means of electrophoreograms. The dog serum before and after injection of emulsions was obtained through the cooperation of the Department of Surgery of the Louisiana State University Medical School. Electrophoreograms of the serum of several dogs before the infusion of emulsions and at several intervals of time after the infusions were compared with the electrophoreograms of the serum from the fasting dogs and with the "in vitro" mixtures of the serum and emulsions.

The electrophoreograms of each mixture of serum and emulsion were made simultaneously with the electrophoreogram of serum alone as the control. The oiled strips were scanned by means of a photoelectric densiotometer which had an automatic integrator. The relative percentage and electrophoretic mobility of each serum protein fraction and the lipids were calculated. Changes due to various emulsifiers were noted.

Results: The pattern of the albumin fraction and its mobility were essentially the same in the serum - emulsion mixtures as in the serum alone. However, when unemulsified cottonseed oil was added to the serum, the mobility of the albumin fraction was decreased.

The most significant observation was that the $alpha_1$ fraction was notably absent in all cases where the emulsion in the serum - emulsion mixtures contained soybean phosphatide as the emulsifier. In all other serum - emulsion mixtures the $alpha_1$ globulin pattern was essentially unaltered as to mobility. When cottonseed oil was added to the serum, the mobility of the $alpha_1$ fraction decreased. However, if the oil was first emulsified, the $alpha_1$ fraction remained the same as in the serum alone. The $alpha_2$, beta and gamma globulin fractions moved with essentially the same mobilities in all mixtures. The mobility of the fat in the serum was between the mobilities of the beta and $alpha_2$ fractions. In all mixtures of serum and emulsions except in those emulsions containing soybean phosphatides, the fat occurred between the beta and $alpha_2$ fractions. In some cases in which the emulsions contained soybean phosphatides, the fat moved with a greater mobility than the $alpha_2$ fraction of that serum - emulsion mixture. The emulsions containing soybean phosphatides also remove the $alpha_2$ fraction from the rat serum. It is interesting to note that emulsion 222-15W, which was toxic to rats, also removed the $alpha_1$ fraction from the rat serum. There are not enough data with rat serum at present to draw conclusions, however.

Experiments on the "in vivo" and "in vitro" mixtures of dog serum with phosphatide and non-phosphatide emulsions are now in progress.

An abstract of a manuscript entitled "Fat Emulsions. Effect on Serum Proteins" is to be included in the program of the 40th Annual Meeting of the Federation of American Societies for Experimental Biology, Atlantic City, New Jersey, April 16-20, 1956.

REFERENCES

(1) D. J. Hanahan, J. Biol. Chem. 206, 443(1954).

(2) J. W. Dieckert and R. Reiser, Fed. Proc. 14, 202(1955).

(3) Ruth R. Benerito, Katherine M. Formusa, J. L. White, and W. S. Singleton, "The Browning Reaction in Phosphatide-Containing Fat Emulsions for Intravenous Alimentation," in manuscript.

VII. CRITICAL REVIEW OF STATUS AND PROSPECTS

1. As yet there does not seem to be a practical fat emulsion which can be used routinely for intravenous alimentation. This fact was made clear at the last meeting of the Task Force on Intravenous Emulsions held in September 1955 and no new information has appeared since which would reflect a change in the status.

2. There is no reason to believe that the need for such an emulsion for use in treatment of military or civilian medical patients has decreased. On the contrary, continued discussions with medical authorities have emphasized the great potential value of such an emulsion if it were available.

3. There seems to be no advantage to varying the oil used in the preparation of the emulsion. Commercial cottonseed oil prepared under the specifications of the Southern Regional Research Laboratory has performed satisfactorily. Use of this oil in further experimentation would eliminate, for the time being, one variable in the test. When other more critical problems are solved it might be worthwhile to return to the question of the oil to determine whether any further improvements could be made in the oil. In the meantime, however, cottonseed oil would seem to be a satisfactory oil for continued experimentation.

4. Practically all of the emulsions made today have had phosphatide as an essential component of the emulsifier system. We have every reason to believe that the purity and the composition of the phosphatide varies depending on the history and processing of the source material and on the conditions of purification of the phosphatide. Even the so-called highly purified phosphatides used in making some of the preparations that were tested contained quite a number of components. It would seem that considerable effort should be expended on determining the purity of the various preparations, improving their purity, evaluating their stability, and if possible, determining the behavior of emulsions prepared when very pure phosphatides are used. Unless work of this type is continued, and possibly expanded, it is difficult to see how one can approach the problem of using phosphatides as part of the emulsion system with any degree of security.

5. The difficulties experienced with emulsions containing phosphatides as part of the emulsifier system, have directed attention to using emulsion systems which do not have these materials as ingredients. We have been successful in making several of these systems, some of which have passed the first animal tests to demonstrate levels of toxicity. This work should be accelerated, particularly the testing, so as to explore fully the possibilities inherent in this type of emulsion. We have received assurances from manufacturers of the most promising commercial emulsifiers that they will cooperate with us fully and completely in trying to work out means of producing materials of uniform quality.

6. Work on these emulsions would be accelerated if means were available for assaying the reactions in the blood when the emulsion is mixed with the blood constituents. We have made considerable progress in determining

the protein and fat pattern in blood by use of paper electrophoresis. This seems to offer promise as a screening technique for determining the differences between various emulsion systems particularly for trying to correlate physiological reactions with such physico-chemical properties as can be measured by this kind of a system. This work should be continued and should be closely correlated with physiological and clinical testing.

7. There is need for more information on what actually happens in the animal system when emulsions are administered. More pharmacological and physiological work correlated and coordinated with the chemical and the in vitro work is essential to the success of the program.

8. The difficulties occasioned by the use of the present emulsions have led many to believe that the solution to the problem lies in the formation of homogeneous high caloric systems involving some sort of soluble fat combinations. We do not believe that every possibility for use of emulsions has been exhausted. On the contrary, we have a strong feeling of optimism that the present work will lead to the solution of the problem. The use of soluble materials which would have lower caloric value than the emulsions but would have the advantage of being homogeneous cannot, however, be overlooked and we are studying a number of suggestions made to us. We are not yet in a position to offer comment on their possibilities.

9. Although the development of fat emulsions suitable for routine intravenous alimentation is a very practical problem, its solution will depend on the development of a considerable amount of background information in chemistry, in emulsion technology, physiology, and pharmacology. A considerable amount of this information necessary for the solution of the practical problem is not yet available. Our efforts have been directed at trying to build up a fund of this systematic information which we believe, in the last analysis, will be the most rapid approach to practical solution of the problems.

VIII. PUBLICATIONS - REPORTS

The research on fat emulsions at the Southern Regional Research Laboratory has led to several complete units of work which were prepared as manuscripts for publication. It is expected that these publications will be of interest and use to others in the field of fat emulsions.

Several of these publications have been, or will be presented at National Meetings and advantage taken of the opportunity to discuss fat emulsions with other investigators in this and allied fields.

The list of publications follow:

1. Surface and Interfacial Tensions of Synthetic Glycerides of Known Composition and Configuration -- Ruth R. Benerito, W. S. Singleton, and R. O. Feuge, J. Phys. Chem. 58, 831 (1954). (Presented at twenty-eighth National Colloid Symposium.)

2. Surface Phenomena of Fats for Parenteral Nutrition -- W. S. Singleton and Ruth R. Benerito, J. Am. Oil Chemists' Soc. 32, 23 (1955). (Presented at 44th annual meeting of American Society of Biological Chemists.)

3. The Preparation and Properties of Alpha, Gamma-dipalmito-beta-lactin -- Leo A. Goldblatt, David A. Yeadon, and Mona Brown, J. Am. Chem. Soc. 77, 2477 (1955). (Presented at Spring Meeting of the American Chemical Society, 1954.)

4. The Browning Reaction in Phosphatide-Containing Fat Emulsions -- Ruth R. Benerito, Katherine M. Formusa, J. L. White, and W. S. Singleton, in manuscript.

5. Fat Emulsions. Effect of Heat on Solubility of Hydrophilic Emulsifiers. Ruth R. Benerito and W. S. Singleton. (Submitted to J. Am. Oil Chemists' Soc. for publication.)

6. Fat Emulsions. Effects on Serum Proteins -- Ruth R. Benerito, Katherine M. Formusa, W. S. Singleton, and J. L. White. (Submitted to Fed. Proc. 1956. To be presented at meeting of Federation of Biological Societies, April, 1956.)

7. A Survey on Phosphatides as Applicable to Fat Emulsions for Intravenous Alimentation -- David A. Yeadon (mimeographed).

8. Preparation & Properties of α-Palmityl-β,γ-Dilactin -- D. A. Yeadon, Mona Brown, and L. A. Goldblatt, in manuscript.

9. Chromatography of the Phospholipids and Related Substances on Glass Paper Impregnated with Silicic Acid. --- Mona Brown, D. A. Yeadon, L. A. Goldblatt and J. W. Dieckert. To be presented at the 129th meeting of the Am. Chem. Soc., Dallas, Texas, April 8-13, 1956.

There have been 12 quarterly reports to the Office of Surgeon General, giving the progress of the emulsion program at the Southern Regional Research Laboratory.